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<b>(21) International Application Number:</b> PCT/GB99/02444 <b>(22) International Filing Date:</b> 27 July 1999 (27.07.99)  <b>(30) Priority Data:</b> 9816335.5                      27 July 1998 (27.07.98)                      GB 60/125,163                      19 March 1999 (19.03.99)                      US  <b>(71) Applicant (for all designated States except US):</b> MICROBIAL TECHNIQS LIMITED [GB/GB]; 20 Trumpington Street, Cambridge CB2 1QA (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LE PAGE, Richard, William, Falla [GB/GB]; University of Cambridge, Dept. of Pathology, Tennis Court Road, Cambridge CB2 1QP (GB). WELLS, Jeremy, Mark [GB/GB]; Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA (GB). HANNIFFY, Sean, Bosco [IE/GB]; University of Cambridge, Dept. of Pathology, Tennis Court Road, Cambridge CB2 1QP (GB).  <b>(74) Agents:</b> CHAPMAN, Paul, William et al.; Kilburn & Strode, 20 Red Lion Street, London WC1R 4PJ (GB).	<b>(81) Designated States:</b> CA, CN, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>	
<b>(54) Title:</b> NUCLEIC ACIDS AND PROTEINS FROM GROUP B STREPTOCOCCUS		
<b>(57) Abstract</b>  Novel protein antigens from Group B <i>Streptococcus</i> are described, together with nucleic acid sequences encoding them. Their use in vaccines and screening methods is also described.		

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### NUCLEIC ACIDS AND PROTEINS FROM GROUP B STREPTOCOCCUS

The present invention relates to proteins derived from *Streptococcus agalactiae*, nucleic acid molecules encoding such proteins, and the use of the proteins as antigens and/or immunogens and in detection/diagnosis. It also relates to a method for the rapid screening of bacterial genomes to isolate and characterise bacterial cell envelope associated or secreted proteins.

The *Group B Streptococcus* (GBS) (*Streptococcus agalactiae*) is an encapsulated bacterium which emerged in the 1970s as a major pathogen of humans causing sepsis and meningitis in neonates as well as adults. The incidence of early onset neonatal infection during the first 5 days of life varies from 0.7 to 3.7 per 1000 live births and causes mortality in about 20% of cases. Between 25-50% of neonates surviving early onset infections frequently suffer neurological sequelae. Late onset neonatal infections occur from 6 days to three months of age at a rate of about 0.5 - 1.0 per 1000 live births.

There is an established association between the colonisation of the maternal genetic tract by GBS at the time of birth and the risk of neonatal sepsis. In humans it has been established that the rectum may act as a reservoir for GBS. Susceptibility in the neonate is correlated with the a low concentration or absence of IgG antibodies to the capsular polysaccharides found on GBS causing human disease. In the USA strains isolated from clinical cases usually belong to capsular serotypes Ia, Ib, II, III although serotype V may be of increasing significance. Type VIII GBS is the major cause of neonatal sepsis in Japan.

A possible means of prevention involves intra or postpartum administration of antibiotics to the mother but there are concerns that this might lead to the emergence of resistant organisms and in some cases allergic reactions. Vaccination of the adolescent females to induce long lasting maternally derived immunity is one of the most promising approaches to prevent GBS infections in neonates. The capsular

polysaccharide antigens of these organisms have attracted most attention as with regard to vaccine development. Studies in healthy adult volunteers have shown that serotype 1a, II and III polysaccharides are non-toxic and immunogenic in approximately 65%, 95% and 70% of non-immune adults respectively. One of the problems with using capsule antigens as vaccines is that the response rates vary according to pre-immunisation status and the polysaccharide antigen and not all vaccinees produce adequate levels of IgG antibody as indicated in vaccination studies with GBS polysaccharides in human volunteers.

Some people do not respond despite repeated stimuli. These properties are due to the T-independent nature of polysaccharide antigens. One strategy to enhance the immunogenicity of these vaccines is to enhance the T cell dependent properties of polysaccharides by conjugating them to a protein. The use of polysaccharide conjugates looks promising but there are still unresolved questions concerning the nature of the carrier protein. A conjugate vaccine against GBS would require at least 4 different conjugates to be prepared adding to the cost of a vaccine.

Recent evidence also suggests that bacterial surface proteins may be useful to confer immunity. A protein called Rib which is found on most serotype III strains but rarely on serotypes Ia, Ib or II confers immunity to challenge with Rib expressing GBS in animal models (Stalhammar-Carlemalm *et al.*, *Journal of Experimental Medicine* 177:1593-1603 (1993)). Another surface protein of interest as a component of a vaccine is the alpha antigen of the C proteins which protected vaccinated mice against lethal infection with strains expressing alpha protein. The amount of antigen expressed by GBS strains varies markedly.

Approaches to vaccination against GBS infections which rely on the use of capsular polysaccharides have the disadvantage that response rates are likely to vary considerably according to pre-immunisation status and the particular type of polysaccharide antigen used. Results of trials in human volunteers have indicated that

response rates may only be around 65% for some of the key capsule antigens (Larsson *et al.*, *Infection and Immunity* 64:3518-3523 (1996)). It is also not clear whether all individuals responding to the vaccine would have adequate levels of polysaccharide specific IgG which can cross the placenta and afford immunity to neonates. By conjugating a protein carrier to the polysaccharide antigen it may be possible to convert them to T-cell dependent antigens and enhance their immunogenicity.

Preliminary studies with GBS type III polysaccharide-tetanus toxoid conjugate have been encouraging (Baker *et al.*, *Reviews of Infectious Diseases* 7:458-467 (1985), Baker *et al.*, *The New England Journal of Medicine* 319:1180-1185 (1988), Paoletti *et al.*, *Infection and Immunity* 64:677-679 (1996), Paoletti *et al.*, *Infection and Immunity* 62:3236-3243 (1994)) but in developed countries the use of tetanus may be disadvantageous since most adults will have been immunised against tetanus within the past five years. Additional boosters with tetanus toxoid may cause adverse reactions (Boyer., *Current Opinions in Pediatrics* 7:13-18 (1995)). The polysaccharide conjugate vaccines have the disadvantage of being costly to produce and manufacture in comparison with many other kinds of vaccines. There is also the possible risk of problems caused by the cross reactivity between GBS polysaccharides and sialic acid-containing human glycoproteins.

An alternative to polysaccharides as antigens is the use of protein antigens derived from GBS. Recent evidence suggest that the GBS surface associated proteins Rib and alpha C protein may be used to confer immunity to GBS infections in experimental model systems (Stalhammar-Carlemalm *et al.*, (1993) [*supra*], Larsson *et al.*, (1996) [*supra*]). However these two proteins are not conserved in all serotypes of GBS which cause disease in humans. Assuming that these antigens would be immunogenic and elicit protective level responses in humans they would not confer protection against all infections as 10% of infectious *Group B streptococci* do not express Rib or C protein alpha.

This invention seeks to overcome the problem of vaccination against GBS by using a novel screening method specifically designed to identify those *Group B Streptococcus* genes encoding bacterial cell surface associated or secreted proteins (antigens). The proteins expressed by these genes may be immunogenic, and therefore may be useful  
5 in the prevention and treatment of *Group B Streptococcus* infection. For the purposes of this application, the term immunogenic means that these proteins will elicit a protective immune response within a subject. Using this novel screening method a number of genes encoding novel *Group B Streptococcus* proteins have been identified.

10 Thus in a first aspect, the present invention provides a *Group B Streptococcus* protein, having a sequence selected from those shown in figure 1, or fragments or derivatives thereof.

15 It will be apparent to the skilled person that proteins and polypeptides included within this group may be cell surface receptors, adhesion molecules, transport proteins, membrane structural proteins, and/or signalling molecules.

20 Alterations in the amino acid sequence of a protein can occur which do not affect the function of a protein. These include amino acid deletions, insertions and substitutions and can result from alternative splicing and/or the presence of multiple translation start sites and stop sites. Polymorphisms may arise as a result of the infidelity of the translation process. Thus changes in amino acid sequence may be tolerated which do not affect the proteins function.

25 Thus, the present invention includes derivatives or variants of the proteins, polypeptides, and peptides of the present invention which show at least 50% identity to the proteins, polypeptides and peptides described herein. Preferably the degree of sequence identity is at least 60% and preferably it is above 75%. More preferably still is it above 80%, 90% or even 95%.

30

The term identity can be used to describe the similarity between two polypeptide sequences. A software package well known in the art for carrying out this procedure is the CLUSTAL program. It compares the amino acid sequences of two polypeptides and finds the optimal alignment by inserting spaces in either sequence as appropriate.

5 The amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment can also be calculated using a software package such as BLASTx. This program aligns the largest stretch of similar sequence and assigns a value to the fit. For any one pattern comparison several regions of similarity may be found, each having a different score. One skilled in the art will appreciate that two  
10 polypeptides of different lengths may be compared over the entire length of the longer fragment. Alternatively small regions may be compared. Normally sequences of the same length are compared for a useful comparison to be made.

15 Manipulation of the DNA encoding the protein is a particularly powerful technique for both modifying proteins and for generating large quantities of protein for purification purposes. This may involve the use of PCR techniques to amplify a desired nucleic acid sequence. Thus the sequence data provided herein can be used to design primers for use in PCR so that a desired sequence can be targeted and then amplified to a high degree.

20 Typically primers will be at least five nucleotides long and will generally be at least ten nucleotides long (e.g. fifteen to twenty-five nucleotides long). In some cases primers of at least thirty or at least thirty-five nucleotides in length may be used.

25 As a further alternative chemical synthesis may be used. This may be automated. Relatively short sequences may be chemically synthesised and ligated together to provide a longer sequence.

Thus in a further aspect, the present invention provides , a nucleic acid molecule comprising or consisting of a sequence which is:

- 5
- (i) any of the DNA sequences set out in figure 1 herein or their RNA equivalents;
  - (ii) a sequence which is complementary to any of the sequences of (i);
  - (iii) a sequence which codes for the same protein or polypeptide, as those sequences of (i) or (ii);
  - (iv) a sequence which shows substantial identity with any of those of (i), (ii) and (iii); or
  - (v) a sequence which codes for a derivative or fragment of a nucleic acid molecule shown in figure 1.

10 The term identity can also be used to describe the similarity between two individual DNA sequences. The 'bestfit' program (Smith and Waterman, *Advances in applied Mathematics*, 482-489 (1981)) is one example of a type of computer software used to find the best segment of similarity between two nucleic acid sequences, whilst the GAP program enables sequences to be aligned along their whole length and finds the  
15 optimal alignment by inserting spaces in either sequence as appropriate.

The term 'RNA equivalent' when used above indicates that a given RNA molecule has a sequence which is complementary to that of a given DNA molecule, allowing for the fact that in RNA 'U' replaces 'T' in the genetic code. The nucleic acid molecule may  
20 be in isolated or recombinant form.

The nucleic acid molecule may be in an isolated or recombinant form. DNA constructs can readily be generated using methods well known in the art. These techniques are disclosed, for example in J. Sambrook *et al*, *Molecular Cloning 2<sup>nd</sup> Edition*, Cold  
25 Spring Harbour Laboratory Press (1989). Modifications of DNA constructs and the proteins expressed such as the addition of promoters, enhancers, signal sequences, leader sequences, translation start and stop signals and DNA stability controlling regions, or the addition of fusion partners may then be facilitated.

5 Normally the DNA construct will be inserted into a vector which may be of phage or plasmid origin. Expression of the protein is achieved by the transformation or transfection of the vector into a host cell which may be of eukaryotic or prokaryotic origin. Such vectors and suitable host cells form yet further aspects of the present invention.

10 The *Group B Streptococcus* proteins (antigens) described herein can additionally be used to raise antibodies, or to generate affibodies. These can be used to detect *Group B Streptococcus*.

Thus in a further aspect the present invention provides, an antibody, affibody, or a derivative thereof which binds to any one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, as described herein.

15 Antibodies within the scope of the present invention may be monoclonal or polyclonal. Polyclonal antibodies can be raised by stimulating their production in a suitable animal host (e.g. a mouse, rat, guinea pig, rabbit, sheep, goat or monkey) when a protein as described herein, or a homologue, derivative or fragment thereof, is injected into the animal. If desired, an adjuvant may be administered together with the protein. Well-  
20 known adjuvants include Freund's adjuvant (complete and incomplete) and aluminium hydroxide. The antibodies can then be purified by virtue of their binding to a protein as described herein.

25 Monoclonal antibodies can be produced from hybridomas. These can be formed by fusing myeloma cells and spleen cells which produce the desired antibody in order to form an immortal cell line. Thus the well-known Kohler & Milstein technique (*Nature* 256 (1975)) or subsequent variations upon this technique can be used.

30 Techniques for producing monoclonal and polyclonal antibodies that bind to a particular polypeptide/protein are now well developed in the art. They are discussed in standard

immunology textbooks, for example in Roitt *et al*, *Immunology* second edition (1989), Churchill Livingstone, London.

5 In addition to whole antibodies, the present invention includes derivatives thereof which are capable of binding to proteins etc as described herein. Thus the present invention includes antibody fragments and synthetic constructs. Examples of antibody fragments and synthetic constructs are given by Dougall *et al* in *Tibtech* 12 372-379 (September 1994).

10 Antibody fragments include, for example, Fab, F(ab')<sub>2</sub> and Fv fragments. Fab fragments (These are discussed in Roitt *et al* [*supra*]). Fv fragments can be modified to produce a synthetic construct known as a single chain Fv (scFv) molecule. This includes a peptide linker covalently joining V<sub>h</sub> and V<sub>l</sub> regions, which contributes to the stability of the molecule. Other synthetic constructs that can be used include CDR peptides. These are  
15 synthetic peptides comprising antigen-binding determinants. Peptide mimetics may also be used. These molecules are usually conformationally restricted organic rings that mimic the structure of a CDR loop and that include antigen-interactive side chains.

20 Synthetic constructs include chimaeric molecules. Thus, for example, humanised (or primatised) antibodies or derivatives thereof are within the scope of the present invention. An example of a humanised antibody is an antibody having human framework regions, but rodent hypervariable regions. Ways of producing chimaeric antibodies are discussed for example by Morrison *et al* in *PNAS*, 81, 6851-6855 (1984) and by Takeda *et al* in *Nature*. 314, 452-454 (1985).

25 Synthetic constructs also include molecules comprising an additional moiety that provides the molecule with some desirable property in addition to antigen binding. For example the moiety may be a label (e.g. a fluorescent or radioactive label). Alternatively, it may be a pharmaceutically active agent.

30

Affibodies are proteins which are found to bind to target proteins with a low dissociation constant. They are selected from phage display libraries expressing a segment of the target protein of interest (Nord K, Gunneriusson E, Ringdahl J, Stahl S, Uhlen M, Nygren PA, Department of Biochemistry and Biotechnology, Royal Institute of Technology (KTH), Stockholm, Sweden).

In a further aspect the invention provides an immunogenic composition comprising one or more proteins, polypeptides, peptides, fragments or derivatives thereof, or nucleotide sequences described herein. A composition of this sort may be useful in the treatment or prevention of *Group B Streptococcus* infection in subject. In a preferred aspect of the invention the immunogenic composition is a vaccine.

In other aspects the invention provides:

- i) Use of an immunogenic composition as described herein in the preparation of a medicament for the treatment or prophylaxis of *Group B Streptococcus* infection. Preferably the medicament is a vaccine.
- ii) A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one antibody, affibody, or a derivative thereof, as described herein.
- iii) A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one protein, polypeptide, peptide, fragments or derivatives as described herein.
- iv) A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one nucleic acid molecule as described herein.

- v) A kit for the detection of *Group B Streptococcus* comprising at least one antibody, affibody, or derivatives thereof, described herein.
- 5 vi) A kit for the detection of *Group B Streptococcus* comprising at least one *Group B Streptococcus* protein, polypeptide, peptide, fragment or derivative thereof, as described herein.
- vii) A kit for the detection of *Group B Streptococcus* comprising at least one nucleic acid of the invention.
- 10

As described previously, the novel proteins described herein are identified and isolated using a novel screening method which specifically identifies those *Group B Streptococcus* genes encoding bacterial cell envelope associated or secreted proteins.

- 15 The information necessary for the secretion/export of proteins has been extensively studied in bacteria. In the majority of cases, export requires a signal peptide positioned at the N-terminus of the precursor protein to target the precursor to translocation sites on the membrane. During or after translocation, the signal peptide is removed by a signal peptidase. The ultimate destination/localisation of the protein, (whether it be
- 20 secreted extracellularly or anchored to the bacterium's surface, etc) is determined by sequences other than the leader peptide sequence.

25 Recently, Poquet *et al.* (*J. Bacteriol.* 180:1904-1912 (1998)) have described a screening vector incorporating the *nuc* gene lacking its own signal leader as a reporter to identify exported proteins in Gram positive bacteria, and have applied it to *L. lactis*. Staphylococcal nuclease is a naturally secreted heat-stable, monomeric enzyme which has been efficiently expressed and secreted in a range of Gram positive bacteria (Shortle., *Gene* 22:181-189 (1983), Kovacevic *et al.*, *J. Bacteriol.* 162:521-528 (1985), Miller *et al.*, *J. Bacteriol.* 169:3508-3514 (1987), Liebl *et al.*, *J. Bacteriol.*

174:1854-1861(1992), Le Loir *et al.*, *J. Bacteriol.* 176:5135-5139 (1994), Poquet *et al.*, 1998 [*supra*]). The screening vector (pFUN) contains the pAM $\beta$ 1 replicon which functions in a broad host range of Gram-positive bacteria in addition to the ColE1 replicon that promotes replication in *Escherichia coli* and certain other Gram  
5 negative bacteria. Unique cloning sites present in the vector can be used to generate transcriptional and translational fusions between cloned genomic DNA fragments and the open reading frame of the truncated *nuc* gene devoid of its own signal secretion leader. The *nuc* gene makes an ideal reporter gene because the secretion of nuclease can readily be detected using a simple and sensitive plate test: Recombinant colonies  
10 secreting the nuclease develop a pink halo whereas control colonies remain white (Shortle, 1983 [*supra*], Le Loir *et al.*, 1994 [*supra*]).

A direct screen to identify and isolate DNA encoding bacterial cell envelope associated or secreted proteins (antigens).in pathogenic bacteria has been developed by  
15 the present inventors which utilises a vector-system (pTREP1 expression vector) in *Lactococcus lactis* that specifically detects DNA sequences which are adjacent to, and associated with DNA encoding surface proteins from *Group B Streptococcus*. The screening vector also incorporates the *nuc* gene encoding the *Staphylococcal* nuclease as a reporter gene.

20 Only the part of the *nuc* gene encoding the mature nuclease protein (minus its signal peptide sequence) is cloned into the pTREP1 expression vector in *L. lactis*. In this form, the *nuc*-encoded nuclease cannot be secreted even when expressed intracellularly. The reporter vector is then randomly combined with appropriately  
25 digested genomic DNA from *Group B Streptococcus*, cloned into *L. lactis* and used as a screening system for sequences permitting the export of nuclease. In this way gene/partial gene sequences encoding exported proteins from *Group B Streptococcus* are isolated. Once a partial gene sequence is obtained, full length sequences encoding exported proteins can readily be obtained using techniques well known in the art.

5 In possessing a promoter, the pTREP1-*nuc* vectors differ from the pFUN vector described by Poquet *et al.* (1998) [*supra*], which was used to identify *L. lactis* exported proteins by screening directly for *Nuc* activity directly in *L. lactis*. As the  
10 pFUN vector does not contain a promoter upstream of the *nuc* open reading frame the cloned genomic DNA fragment must also provide the signals for transcription in addition to those elements required for translation initiation and secretion of *Nuc*. This limitation may prevent the isolation of genes that are distant from a promoter for example genes which are within polycistronic operons. Additionally there can be no  
15 guarantee that promoters derived from other species of bacteria will be recognised and functional in *L. lactis*. Certain promoters may be under stringent regulation in the natural host but not in *L. lactis*. In contrast, the presence of the P1 promoter in the pTREP1-*nuc* series of vectors ensures that promoterless DNA fragments (or DNA fragments containing promoter sequences not active in *L. lactis*) may still be  
transcribed. Thus yet another advantage of this invention is that genes missed in other screening methods may be identified.

Hence in a further aspect the present invention provides a method of screening for DNA encoding bacterial cell wall associated or surface antigens in gram positive  
20 bacteria comprising the steps of:

- combining a reporter vector including the nucleotide sequence encoding the mature form of the staphylococcus nuclease gene and an upstream promoter region with DNA from a gram positive bacteria.
- transforming the resultant vector into *Lactococcus lactis* cells.
- 25 - assaying for the secretion of *staphylococcus* nuclease protein in the transformed cells.

Preferably, the reporter vector is one of the pTREP1-*nuc* vectors shown in figure 4.

In another aspect, the present invention provides a vector as shown in figure 4 for use in screening for DNA encoding exported or surface antigens in gram positive bacteria. Examples of gram positive bacteria which may be screened include *Group B Streptococcus*, *Streptococcus pneumoniae*, *Staphylococcus aureus* or pathogenic  
5 *Group A Streptococci*.

Given that the inventors have identified a group of important proteins, such proteins are potential targets for anti-microbial therapy. It is necessary, however, to determine whether each individual protein is essential for the organism's viability.  
10 Thus, the present invention also provides a method of determining whether a protein or polypeptide as described herein represents a potential anti-microbial target which comprises inactivating said protein and determining whether *Group B Streptococcus* is still viable.

15 A suitable method for inactivating the protein is to effect selected gene knockouts, ie prevent expression of the protein and determine whether this results in a lethal change. Suitable methods for carrying out such gene knockouts are described in Li *et al*, *P.N.A.S.*, 94:13251-13256 (1997) and Kolkman *et al*

20 In a final aspect the present invention provides the use of an agent capable of antagonising, inhibiting or otherwise interfering with the function or expression of a protein or polypeptide of the invention in the manufacture of a medicament for use in the treatment or prophylaxis of *Group B Streptococcus* infection.

25 The invention will now be described by means of the following example which should not in any way be construed as limiting. The examples refer to the figures in which

Fig 1: (A) Shows a number of full length nucleotide sequences encoding antigenic *Group B Streptococcus* proteins. (B) Shows the corresponding amino acid sequences.

5 Fig 2: Shows a number of oligonucleotide primers used in the screening process

**nucS1** primer designed to amplify a mature form of the nuc A gene

**nucS2-** primer designed to amplify a mature form of the nuc A gene.

**nucS3** primer designed to amplify a mature form of the nuc A gene

10 **nucR** primer designed to amplify a mature form of the nuc A gene

**nucseq** primer designed to sequence DNA cloned into the pTREP-Nuc vector  
**pTREPF** nucleic acid sequence containing recognition site for ECORV. Used for cloning fragments into pTREX7.

15 **pTREPR** nucleic acid sequence containing recognition site for BAMH1. Used for cloning fragments into pTREX7.

**PUCF** forward sequencing primer, enables direct sequencing of cloned DNA fragments.

**VR** example of gene specific primer used to obtain further antigen DNA sequence by the method of DNA walking.

20 **V1** example of gene specific primer used to obtain further antigen DNA sequence by the method of DNA walking.

**V2** example of gene specific primer used to obtain further antigen DNA sequence by the method of DNA walking.

25

Fig 3: (i) Schematic presentation of the nucleotide sequence of the unique gene cloning site immediately upstream of the mature *nuc* gene in pTREP1-*nuc1*, pTREP1-*nuc2* and pTREP1-*nuc3*. Each of the pTREP-*nuc* vectors contain an

EcoRV (a SmaI site in pTREP1-*nuc2*) cleavage site which allows cloning of genomic DNA fragments in 3 different frames with respect to the mature *nuc* gene.

(ii) A physical and genetic summary map of the pTREP1-*nuc* vectors. The expression cassette incorporating *nuc*, the macrolides, lincosamides and streptogramin B (MLS) resistance determinant, and the replicon (rep) *Ori-pAMβ1* are depicted (not drawn to scale).

(iii) Schematic presentation of the expression cassette showing the various sequence elements involved in gene expression and location of unique restriction endonuclease sites (not drawn to scale).

Fig 4: Shows the results of various DNA vaccine trials;

Fig 5: Shows the results of a second group of DNA vaccine trials;

Figs 6-11: Show various Southern Blot analyses of different Group B streptococcus strains.

#### Example 1

Thus far more than 100 gene/partial gene sequences putatively encoding exported proteins in *S. agalactiae* have been identified using the nuclease screening system of the invention. These have been further analysed to remove artifacts. The nucleotide sequences of genes identified using the screening system has been characterised using a number of parameters described below. All of these sequences are novel in that they have not been described previously.

1. All putative surface proteins are analysed for leader/signal peptide sequences. Bacterial signal peptide sequences share a common design. They are characterised by a short positively charged N-terminus (N region) immediately preceding a stretch of hydrophobic residues (central portion-h region) followed by a

more polar C-terminal portion which contains the cleavage site (c-region). Computer software is used to perform hydropathy profiling of putative proteins (Marcks, *Nuc. Acid. Res.*, 16:1829-1836 (1988)) which is used to identify the distinctive hydrophobic portion (h-region) typical of leader peptide sequences. In addition, the presence/absence of a potential ribosomal binding site (Shine-Dalgarno sequence required for translation) is also noted.

2. All putative surface protein sequences are used to search the OWL sequence database which includes a translation of the GENBANK and SWISSPROT database.. This allows identification of similar sequences which may have been previously characterised not only at the sequence level but at a functional level. It may also provide information indicating that these proteins are indeed surface related and not artifacts.

3. Putative *S. agalactiae* surface proteins are also be assessed for their novelty. Some of the identified proteins may or may not possess a typical leader peptide sequence and may not show homology with any DNA/protein sequences in the database. Indeed these proteins may indicate the primary advantage of our screening method, i.e. isolating atypical surface-related proteins, which would have been missed in all previously described screening protocols.

The construction of three reporter vectors and their use in *L. lactis* to identify and isolate genomic DNA fragments from pathogenic bacteria encoding secreted or surface associated proteins is now described.

## **Construction of the pTREP1-nuc series of reporter vectors**

### **(a) Construction of expression plasmid pTREP1**

The pTREP1 plasmid is a high-copy number (40-80 per cell) theta-replicating gram positive plasmid, which is a derivative of the pTREX plasmid which is itself a derivative of the the previously published pIL253 plasmid. pIL253 incorporates the

broad Gram-positive host range replicon of pAM $\beta$ 1 (Simon and Chopin, 1988) and is non-mobilisable by the *L. lactis* sex-factor. pIL253 also lacks the *tra* function which is necessary for transfer or efficient mobilisation by conjugative parent plasmids exemplified by pIL501. The Enterococcal pAM $\beta$ 1 replicon has previously been transferred to various species including *Streptococcus*, *Lactobacillus* and *Bacillus* species as well as *Clostridium acetobutylicum*, (LeBlanc *et al.*, *Proceedings of the National Academy of Science USA* 75:3484-3487 (1978)) indicating the potential broad host range utility. The pTREP1 plasmid represents a constitutive transcription vector.

The pTREX vector was constructed as follows. An artificial DNA fragment containing a putative RNA stabilising sequence, a translation initiation region (TIR), a multiple cloning site for insertion of the target genes and a transcription terminator was created by annealing 2 complementary oligonucleotides and extending with Tfl DNA polymerase. The sense and anti-sense oligonucleotides contained the recognition sites for NheI and BamHI at their 5' ends respectively to facilitate cloning. This fragment was cloned between the XbaI and BamHI sites in pUC19NT7, a derivative of pUC19 which contains the T7 expression cassette from pLET1 (Wells *et al.*, *J. Appl. Bacteriol.* 74:629-636 (1993)) cloned between the EcoRI and HindIII sites. The resulting construct was designated pUCLEX. The complete expression cassette of pUCLEX was then removed by cutting with HindIII and blunting followed by cutting with EcoRI before cloning into EcoRI and SacI (blunted) sites of pIL253 to generate the vector pTREX (Wells and Schofield, *In Current advances in metabolism, genetics and applications-NATO ASI Series. H* 98:37-62. (1996)). The putative RNA stabilising sequence and TIR are derived from the *Escherichia coli* T7 bacteriophage sequence and modified at one nucleotide position to enhance the complementarity of the Shine Dalgarno (SD) motif to the ribosomal 16s RNA of *Lactococcus lactis* (Schofield *et al.* pers. coms. University of Cambridge Dept. Pathology.).

5 A *Lactococcus lactis* MG1363 chromosomal DNA fragment exhibiting promoter activity which was subsequently designated P7 was cloned between the EcoRI and BglII sites present in the expression cassette, creating pTREX7. This active promoter region had been previously isolated using the promoter probe vector pSB292 (Waterfield *et al.*, *Gene* 165:9-15 (1995)). The promoter fragment was amplified by PCR using the Vent DNA polymerase according to the manufacturer.

10 The pTREP1 vector was then constructed as follows. An artificial DNA fragment which included a transcription terminator, the forward pUC sequencing primer, a promoter multiple cloning site region and a universal translation stop sequence was created by annealing two overlapping partially complementary synthetic oligonucleotides together and extending with sequenase according to manufacturers instructions. The sense and anti-sense (pTREP<sub>F</sub> and pTREP<sub>R</sub>) oligonucleotides contained the recognition sites for EcoRV and BamHI at their 5' ends respectively to  
15 facilitate cloning into pTREX7. The transcription terminator was that of the *Bacillus penicillinase* gene, which has been shown to be effective in *Lactococcus* (Jos *et al.*, *Applied and Environmental Microbiology* 50:540-542 (1985)). This was considered necessary as expression of target genes in the pTREX vectors was observed to be leaky and is thought to be the result of cryptic promoter activity in the origin region  
20 (Schofield *et al.* pers. coms. University of Cambridge Dept. Pathology.). The forward pUC primer sequencing was included to enable direct sequencing of cloned DNA fragments. The translation stop sequence which encodes a stop codon in 3 different frames was included to prevent translational fusions between vector genes and cloned DNA fragments. The pTREX7 vector was first digested with EcoRI and blunted using  
25 the 5' - 3' polymerase activity of T4 DNA polymerase (NEB) according to manufacturer's instructions. The EcoRI digested and blunt ended pTREX7 vector was then digested with Bgl II thus removing the P7 promoter. The artificial DNA fragment derived from the annealed synthetic oligonucleotides was then digested with EcoRV and Bam HI and cloned into the EcoRI(blunted)-Bgl II digested pTREX7 vector to

generate pTREP. A *Lactococcus lactis* MG1363 chromosomal promoter designated P1 was then cloned between the EcoRI and BglII sites present in the pTREP expression cassette forming pTREP1. This promoter was also isolated using the promoter probe vector pSB292 and characterised by Waterfield *et al.*, (1995) [*supra*]. The P1 promoter fragment was originally amplified by PCR using vent DNA polymerase according to manufacturers instructions and cloned into the pTREX as an EcoRI-BglII DNA fragment. The EcoRI-BglII P1 promoter containing fragment was removed from pTREX1 by restriction enzyme digestion and used for cloning into pTREP (Schofield *et al.* pers. coms. University of Cambridge, Dept. Pathology.).

**(b) PCR amplification of the *S. aureus* nuc gene.**

The nucleotide sequence of the *S. aureus* nuc gene (EMBL database accession number V01281) was used to design synthetic oligonucleotide primers for PCR amplification. The primers were designed to amplify the mature form of the nuc gene designated nucA which is generated by proteolytic cleavage of the N-terminal 19 to 21 amino acids of the secreted propeptide designated Snase B (Shortle, 1983 [*supra*]). Three sense primers (nucS1, nucS2 and nucS3, shown in figure 3) were designed, each one having a blunt-ended restriction endonuclease cleavage site for EcoRV or SmaI in a different reading frame with respect to the nuc gene. Additionally BglII and BamHI were incorporated at the 5' ends of the sense and anti-sense primers respectively to facilitate cloning into BamHI and BglII cut pTREP1. The sequences of all the primers are given in figure 3. Three nuc gene DNA fragments encoding the mature form of the nuclease gene (NucA) were amplified by PCR using each of the sense primers combined with the anti-sense primer. The nuc gene fragments were amplified by PCR using *S. aureus* genomic DNA template, Vent DNA Polymerase (NEB) and the conditions recommended by the manufacturer. An initial denaturation step at 93°C for 2 min was followed by 30 cycles of denaturation at 93°C for 45 sec, annealing at 50°C for 45 seconds, and extension 73°C for 1 minute and then a final 5 min extension step

at 73°C. The PCR amplified products were purified using a Wizard clean up column (Promega) to remove unincorporated nucleotides and primers.

**(c) Construction of the pTREP1-*nuc* vectors**

5 The purified *nuc* gene fragments described in section b were digested with Bgl II and BamHI using standard conditions and ligated to BamHI and BglII cut and dephosphorylated pTREP1 to generate the pTREP1-*nuc*1, pTREP1-*nuc*2 and pTREP1-*nuc*3 series of reporter vectors. These vectors are described in figure 4.

10 General molecular biology techniques were carried out using the reagents and buffers supplied by the manufacturer or using standard techniques (Sambrook and Maniatis, Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press: Cold Spring Harbour (1989)). In each of the pTREP1-*nuc* vectors the expression cassette comprises a transcription terminator, lactococcal promoter P1, unique cloning sites

15 (BglII, EcoRV or SmaI) followed by the mature form of the *nuc* gene and a second transcription terminator. Note that the sequences required for translation and secretion of the *nuc* gene were deliberately excluded in this construction. Such elements can only be provided by appropriately digested foreign DNA fragments (representing the target bacterium) which can be cloned into the unique restriction sites present

20 immediately upstream of the *nuc* gene.

**(d) Screening for secreted proteins in *Group B Streptococcus*.**

Genomic DNA isolated from and *Group B Streptococcus* (*S. agalactiae*) was digested with the restriction enzyme Tru9I. This enzyme which recognises the sequence 5'-

25 TTAA -3' was used because it cuts A/T rich genomes efficiently and can generate random genomic DNA fragments within the preferred size range (usually averaging 0.5 - 1.0 kb). This size range was preferred because there is an increased probability that the P1 promoter can be utilised to transcribe a novel gene sequence. However, the P1 promoter may not be necessary in all cases as it is possible that many Streptococcal

30 promoters are recognised in *L. lactis*. DNA fragments of different size ranges were

purified from partial Tru9I digests of and *S. agalactiae* genomic DNA. As the Tru 9I restriction enzyme generates staggered ends the DNA fragments had to be made blunt ended before ligation to the EcoRV or SmaI cut pTREP1-*nuc* vectors. This was achieved by the partial fill-in enzyme reaction using the 5'-3' polymerase activity of Klenow enzyme. Briefly Tru9I digested DNA was dissolved in a solution (usually between 10-20  $\mu$ l in total) supplemented with T4 DNA ligase buffer (New England Biolabs; NEB) (1X) and 33  $\mu$ M of each of the required dNTPs, in this case dATP and dTTP. Klenow enzyme was added (1 unit Klenow enzyme (NEB) per  $\mu$ g of DNA) and the reaction incubated at 25°C for 15 minutes. The reaction was stopped by incubating the mix at 75°C for 20 minutes. EcoRV or SmaI digested pTREP-*nuc* plasmid DNA was then added (usually between 200-400 ng). The mix was then supplemented with 400 units of T4 DNA ligase (NEB) and T4 DNA ligase buffer (1X) and incubated overnight at 16°C. The ligation mix was precipitated directly in 100% Ethanol and 1/10 volume of 3M sodium acetate (pH 5.2) and used to transform *L. lactis* MG1363 (Gasson, *J. Bacteriol.* 154:1-9 (1983)). Alternatively, the gene cloning site of the pTREP-*nuc* vectors also contains a BglII site which can be used to clone for example Sau3AI digested genomic DNA fragments.

*L. lactis* transformant colonies were grown on brain heart infusion agar and nuclease secreting (*Nuc*<sup>+</sup>) clones were detected by a toluidine blue-DNA-agar overlay (0.05 M Tris pH 9.0, 10 g of agar per litre, 10 g of NaCl per liter, 0.1 mM CaCl<sub>2</sub>, 0.03% wt/vol. salmon sperm DNA and 90 mg of Toluidine blue O dye) essentially as described by Shortle, 1983 [*supra*], and Le Loir *et al.*, 1994 [*supra*]). The plates were then incubated at 37°C for up to 2 hours. Nuclease secreting clones develop an easily identifiable pink halo. Plasmid DNA was isolated from *Nuc*<sup>+</sup> recombinant *L. lactis* clones and DNA inserts were sequenced on one strand using the *NucSeq* sequencing primer described in figure 3, which sequences directly through the DNA insert.

Whilst the example described above related specifically to *Group B Streptococcus*, it will be apparent to one skilled in the art that the same screening technique may be used to detect exported and secreted proteins in other gram positive bacteria, for example *Streptococcus pneumoniae*.

5     **Example 2; Screening Group B Streptococcal derived genes in DNA vaccination experiments.**

**pcDNA3.1+ as a DNA vaccine vector**

10     The commercially available pcDNA3.1+ plasmid (Invitrogen), referred to as pcDNA3.1 henceforth, was used as a vector in all DNA immunisation experiments involving gene targets derived using the LEEP system. pcDNA 3.1 is designed for high-level stable and transient expression in mammalian cells and has been used widely and successfully as a host vector to test candidate genes from a variety of pathogens in DNA vaccination experiments (Zhang *et al.*, 1997; Kurar and Splitter, 15     1997; Anderson *et al.*, 1996).

20     The vector possesses a multiple cloning site which facilitates the cloning of multiple gene targets downstream of the human cytomegalovirus (CMV) immediate-early promoter/enhancer which permits efficient, high-level expression of the target gene in a wide variety of mammalian cells and cell types including both muscle and immune cells. This is important for optimal immune response as it remains unknown as to which cells types are most important in generating a protective response *in vivo*. The plasmid also contains the ColE1 origin of replication which allows convenient high-copy number replication and growth in *E. coli* and the ampicillin resistance gene (B-lactamase) for selection in *E. coli*. In addition pcDNA 3.1 possesses a T7 25     promoter/priming site upstream of the MCS which allows for *in vitro* transcription of a cloned gene in the sense orientation.

30     **Preparation of DNA vaccines**

Oligonucleotide primers were designed for each individual gene of interest derived using the LEEP system. Each gene was examined thoroughly, and where possible, primers were designed such that they targeted that portion of the gene thought to

5 encode only the mature portion of the protein (**APPENDIX 1**). It was hoped that  
expressing those sequences that encode only the mature portion of a target gene  
protein, would facilitate its correct folding when expressed in mammalian cells. For  
example, in the majority of cases primers were designed such that putative N-terminal  
10 signal peptide sequences would not be included in the final amplification product to be  
cloned into the pcDNA3.1 expression vector. The signal peptide directs the  
polypeptide precursor to the cell membrane via the protein export pathway where it is  
normally cleaved off by signal peptidase I (or signal peptidase II if a lipoprotein).  
Hence the signal peptide does not make up any part of the mature protein whether it be  
15 displayed on the bacterium's surface or secreted. Where a N-terminal leader peptide  
sequence was not immediately obvious, primers were designed to target the whole of  
the gene sequence for cloning and ultimately, expression in pcDNA3.1.

20 All forward and reverse oligonucleotide primers incorporated appropriate restriction  
enzyme sites to facilitate cloning into the pcDNA3.1 MCS region. All forward primers  
were also designed to include the conserved Kozak nucleotide sequence 5'-gccacc-3'  
immediately upstream of an 'atg' translation initiation codon in frame with the target  
gene insert. The Kozak sequence facilitates the recognition of initiator sequences by  
eukaryotic ribosomes. Typically, a forward primer incorporating a BamH1 restriction  
25 enzyme site the primer would begin with the sequence 5'-cgggatccgccaccatg-3',  
followed by a sequence homologous to the 5' end of that part of a gene being  
amplified. All reverse primers incorporated a Not I restriction enzyme site sequence 5'-  
ttgcggccgc-3'. All gene-specific forward and reverse primers were designed with  
compatible melting temperatures to facilitate their amplification.

30 All gene targets were amplified by PCR from *S. agalactiae* genomic DNA template  
using Vent DNA polymerase (NEB) or rTth DNA polymerase (PE Applied  
Biosystems) using conditions recommended by the manufacturer. A typical  
amplification reaction involved an initial denaturation step at 95°C for 2 minutes  
followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the  
appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1  
minute per kilobase of DNA being amplified). This was followed by a final extension  
period at 72°C for 10 minutes. All PCR amplified products were extracted once with  
phenol chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated.

Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). The purified amplification gene DNA fragments were digested with the appropriate restriction enzymes and cloned into the pcDNA3.1 plasmid vector using *E. coli* as a host. Successful cloning and maintenance of genes was confirmed by restriction mapping and by DNA sequencing. Recombinant plasmid DNA was isolated on a large scale (>1.5 mg) using Plasmid Mega Kits (Qiagen).

It was decided to include the *S. agalactiae* *rib* gene as a positive control in at least one trial of DNA immunisation experiments. Rabbit antiserum against the Rib protein (Stalhammar-Carleman *et al.*, 1993) and highly purified preparations of the Rib protein itself (Larsson *et al.*, 1999; Larsson *et al.*, 1996) have been shown to confer protection against lethal infection with strains expressing the antigen. All serotype III strains have been shown to express the Rib antigen and Southern blot analysis performed in the laboratory has confirmed that *S. agalactiae* serotype III (strain 97/0099) does contain the *rib* gene, hence the *rib* gene as part of a DNA vaccine would represent a potential positive control for all DNA immunisation experiments. Oligonucleotide primers were designed (**Appendix I**) that targeted only the mature portion of the *rib* gene and which included appropriate restriction enzyme sites for cloning into pcDNA3.1. *rib* was amplified using rTth DNA polymerase (*PE Applied Biosystems*) using conditions recommended by the manufacturer. Conditions for cloning were similar to that described previously.

#### **Preparation of a *S. agalactiae* standard inoculum**

##### **Strain validation**

*S. agalactiae* serotype III (strain 97/0099) is a recent clinical isolate derived from the cerebral spinal fluid of a new born baby suffering from meningitis. This haemolytic strain of Group B Streptococcus was epidemiologically tested and validated at the Respiratory and Systemic Infection Laboratory, PHLS Central Public health laboratory, 61 Collindale Avenue, London NW9 5HT. The strain was subcultured only twice prior to its arrival in the laboratory. Upon its arrival on a agar slope, a sweep of 4-5 colonies was immediately used to inoculate a Todd Hewitt/5% horse blood broth which was incubated overnight statically at 37 °C. 0.5 ml aliquots of this overnight culture were then used to make 20% glycerol stocks of the bacterium for long term

storage at -70 °C. Glycerol stocks were streaked on Todd Hewitt/5% horse blood agar plates to confirm viability.

#### 5 ***In vivo* passaging of Group B Streptococcus**

A frozen culture (described under strain validation) of *S. agalactiae* serotype III (strain 97/0099) was streaked to single colonies on Todd-Hewitt/5% blood agar plates which were incubated overnight at 37°C. A sweep of 4-5 colonies was used to inoculate a Todd Hewitt/5% horse blood broth which was again incubated overnight. A 0.5 ml aliquot from this overnight culture was used to inoculate a 50 ml Todd Hewitt broth (1:100 dilution) which was incubated at 37 °C. 10-fold serial dilutions of the overnight culture were made (since virulence of this strain was unknown) and each were passaged intra-peritoneally (IP) in CBA/ca mice in duplicate. Viable counts were performed on the various inocula used in the passage. Groups of mice were challenged with various concentrations of the pathogen ranging from  $10^8$  to  $10^4$  colony forming units (cfu). Mice that developed symptoms were terminally anaesthetized and cardiac punctures were performed (Only mice that had been challenged with the highest doses, i.e.  $1 \times 10^8$  cfu, developed symptoms). The retrieved unclotted blood was used to inoculate directly a 50ml serum broth (Todd Hewitt/20% inactivated foetal calf serum). The culture was constantly monitored and allowed to grow to late logarithmic phase. The presence of blood in the medium interfered with OD<sub>600</sub> readings as it was being increasingly lysed with increasing growth of the bacterium, hence the requirement to constantly monitor the culture. Upon reaching late logarithmic phase/early stationary phase, the culture was transferred to a fresh 50 ml tube in order to exclude dead bacterial cells and remaining blood cells which would have sedimented at the bottom of the tube. 0.5 ml aliquots were then transferred to sterile cryovials, frozen in liquid nitrogen and stored at -70 °C. A viable count was carried out on a single standard inoculum aliquot in order to determine bacterial numbers. This was determined to be approximately  $5 \times 10^8$  cfu per ml.

#### 30 **Intra-peritoneal Challenge and virulence testing of Group B Streptococcus standard inoculum**

To determine if the standard inoculum was suitably virulent for use in a vaccine trial, challenges were carried out using a dose range. Frozen standard inoculum strain

aliquots were allowed to thaw at room temperature. From viable count data the number of cfu per ml was already known for the standard inoculum. Initially, serial dilutions of the standard inoculum were made in Todd Hewitt broth and mice were challenged intra-peritoneally with doses ranging from  $1 \times 10^8$  to  $1 \times 10^4$  cfu in a 500  $\mu$ l volume of Todd Hewitt broth. The survival times of mouse groups injected with different doses of the bacterium were compared. The standard inoculum was determined to be suitably virulent and a dose of  $1 \times 10^6$  cfu was considered close to optimal for further use in vaccine trials. Further optimisation was carried out by comparing mice challenged with doses ranging between  $5 \times 10^5$  and  $5 \times 10^6$  cfu. The optimal dose was estimated to be approximately  $2.5 \times 10^6$  cfu. This represented a 100% lethal dose and was repeatedly consistent with end-points as determined by survival times being clustered within a narrow time-range. Throughout all these experiments, challenged mice were constantly monitored to clarify symptoms, stages of symptom development as well as calculating survival times.

#### Vaccine trials

Vaccine trials in mice were accomplished by the administration of DNA to 6 week old CBA/ca mice (Harlan, UK). Mice to be vaccinated were divided into groups of six and each group were immunised with recombinant pcDNA3.1 plasmid DNA containing a specific target-gene sequence derived using the LEEP system. A total of 100  $\mu$ g of DNA in Dulbecco's PBS (Sigma) was injected intramuscularly into the tibialis anterior muscle of both hind legs. Four weeks later this procedure was repeated using the same amount of DNA. For comparison, control mice groups were included in all vaccine trials. These control groups were either not DNA-vaccinated or were immunised with non-recombinant pcDNA3.1 plasmid DNA only, using the same time course described above. Four weeks after the second immunisation, all mice groups were challenged intra-peritoneally with a lethal dose of *S. agalactiae* serotype III (strain 97/0099). The actual number of bacteria administered was determined by plating serial dilutions of the inoculum on Todd-Hewitt/5% blood agar plates. All mice were killed 3 or 4 days after infection. During the infection process, challenged mice were monitored for the development of symptoms associated with the onset of *S. agalactiae* induced-disease. Typical symptoms in an appropriate order included piloerection, an increasingly hunched posture, discharge from eyes, increased lethargy and reluctance to move which was often the result of apparent paralysis in the lower body/hind leg region. The

latter symptoms usually coincided with the development of a moribund state at which stage the mice were culled to prevent further suffering. These mice were deemed to be very close to death, and the time of culling was used to determine a survival time for statistical analysis. Where mice were found dead, a survival time was calculated by averaging the time when a particular mouse was last observed alive and the time when found dead, in order to determine a more accurate time of death.

### Interpretation of Results

A positive result was taken as any DNA sequence that was cloned and used in challenge experiments as described above and gave protection against that challenge. DNA sequences were determined to be protective;

-if that DNA sequence gave statistically significant protection (to a 95% confidence level ( $p > 0.05$ ) as determined using the Mann-Whitney U test.

-if that DNA sequence was marginal or non-significant using Mann-Whitney but showed some protective features. For example, one or more outlying mice may survive for significantly longer time periods when compared with control mice. Alternatively, the time to first death may also be prolonged when compared to counterpart mice in control groups.

It is acceptable to allow marginal or non-significant results to be considered as potential positives when it is possible that the clarity of some results may be affected by problems associated with the administration of the DNA vaccine. Indeed, much varied survival times may reflect different levels of immune response between different members of a given group.

### Results

#### Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 1 (Figure 4a)

	Mean Survival Times (hours)				
	pcDNA3.1	17(ID-8)	18(ID-9)	20(ID-25)	rib
1	26.833	14.916	27.750	30.500	88.666

2	42.333	94.000 (T)	34.333	33.333	28.166
3	47.916	45.166	41.083	34.083	37.250
4	28.333	30.750	47.083	23.500	37.250
5	42.333	74.666	94.000 (T)	94.000 (T)	94.000 (T)
6	25.333	25.000	26.166	30.500	45.750
Mean	37.549	51.899	48.849	43.083	57.066
sd	9.3943	32.214	26.257	28.768	31.556
p value 1		0.4049	0.4049	0.5000	0.1481
p value 2	> 39.0	> 39.0	> 39.0	> 39.0	

(T) - terminated at conclusion of experiment but showing symptoms of infection.

5 p value 1 refers to statistical significance when compared to pcDNA3.1 controls.

p value 2 refers to statistical significance when compared to rib positive control.

10

All DNA vaccine's showed a pattern of protection similar to that obtained with the rib DNA vaccine, which was initially used as a positive control.

15

### 17 (ID-8)

20

Mice immunised with the '17 (ID-8)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there are two outlying mice one of which survived the term of the experiment despite developing symptoms. The group also exhibited a much wider range of survival times reflected by a mean survival value which is approximately 14 hours higher than that demonstrated by the unvaccinated control group.

25

### 18 (ID-9)

Mice immunised with the '18 (ID-9)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there is one outlying mouse which survived the term of the experiment despite developing symptoms.

## 20 (ID-25)

Mice immunised with the '20 (ID-25)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there was one outlying mouse which survived the term of the experiment despite developing symptoms.

## 15 Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 2 (Figure 4b)

	Mean Survival Times (hours)			
	pcDNA	UnVacc	22(ID-10)	28(ID-13)
1	45.000	27.916	44.666	72.000 (T)
2	37.333	45.083	51.416	33.000
3	37.333	37.583	40.791	36.083
4	35.291	24.583	44.666	72.000 (T)
5	24.333	37.583	36.916	49.166
6	45.000	33.166	57.833	36.083
Mean	35.858	34.549	43.691	52.449
sd	7.4342	8.2567	5.3825	18.850
p value 1		> 39.0	0.1137	0.2340
p value 2	0.4679		0.0323	0.1137

(T) - terminated at conclusion of experiment but showing symptoms of infection.

p value 1 refers to statistical significance when compared to pcDNA3.1 controls.

p value 2 refers to statistical significance when compared to unvaccinated controls.

5 There was no significant difference in the survival times exhibited by the pcDNA3.1 and unvaccinated control groups. This is confirmed by their very similar mean survival times of 35.858 hours (pcDNA3.1) and 34.166 hours (Unvaccinated).

10

## **22 (ID-10)**

Mice immunised with the '22 (ID-10)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group but not when compared with the pcDNA3.1 control group. In addition, the time to first death in this group was prolonged by approximately 12 hours when compared to the pcDNA3.1 and unvaccinated control groups. The mean survival time of 43.691 hours is also considerably higher than that determined for both control groups.

20

## **28 (ID-13)**

Mice immunised with the '28 (ID-13)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 and unvaccinated control groups. However there are three outlying mice, two of which survived the term of the experiment despite showing symptoms. In addition, the time to first death in this group was prolonged by approximately 9 hours when compared to the pcDNA3.1 and unvaccinated control groups. The mean survival time of 52.449 hours is also considerably higher than that determined for both control groups, as well demonstrating a wider range of survival times.

30

**Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 3 (Figure 4c)**

35

	Mean Survival Times (hours)				
	UnVacc.	70(ID-42)	94(ID-48)	86(ID-47)	51(ID-37)
1	27.583	25.166	34.666	32.416	43.749
2	27.583	42.666	49.500	32.416	38.333
3	24.583	34.666	27.000	42.500	50.666
4	22.250	42.666	30.500	34.500	45.166
5	35.916	30.583	30.500	34.500	69.082
6	22.250	25.166	42.666	42.500	31.166
<b>Mean</b>	<b>27.583</b>	<b>35.149</b>	<b>34.433</b>	<b>35.266</b>	<b>49.399</b>
<b>sd</b>	<b>5.1691</b>	<b>7.6444</b>	<b>8.8495</b>	<b>4.1758</b>	<b>11.846</b>
<b>p value</b>		<b>0.0628</b>	<b>0.0321</b>	<b>0.0153</b>	<b>0.0041</b>

5      **p value** refers to statistical significance when compared to unvaccinated controls.

#### 70 (ID-42)

10      Mice immunised with the '70 (ID-42)' DNA vaccine, marginally did not show significantly longer survival times when compared with the unvaccinated control group. However, the first death in this group is prolonged (by approximately 3 hours ) when compared with the unvaccinated group. In addition, the group has a mean survival time  
15      which is approximately 8 hours longer than the unvaccinated group.

#### 94 (ID-48)

20      Mice immunised with the '94 (ID-48)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

#### 86 (ID-47)

Mice immunised with the '86 (ID-47)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

5

**51 (ID-37)**

Mice immunised with the '51 (ID-37)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

10

**Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 4 (Figure 4d)**

15

	Mean Survival Times (hours)	
	UnVacc	9(ID-6)
1	32.666	35.250
2	21.666	30.958
3	23.916	69.333
4	22.999	52.333
5	25.916	44.916
6	35.916	47.083
Mean	25.432	46.041
sd	4.3291	16.096
p value		0.0101

(T) - terminated at conclusion of experiment but showing symptoms of infection.

20

p value refers to statistical significance when compared to unvaccinated controls

**9 (ID-6)**

Mice immunised with the '39(ID-6)' DNA vaccine showed significantly longer survival times when compared with the control group.

5

### Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 6 (Figure 4e)

10

	Mean Survival Times (hours)				
	pcDNA	UnVacc	32 (ID-15)	39(ID-17)	57(40)
1	33.541	36.000	25.041	52.333	28.333
2	36.750	29.999	30.458	44.750	32.708
3	36.750	32.749	44.833	44.750	36.083
4	36.750	44.500	30.458	36.250	40.333
5	29.000	28.333	64.833	36.250	72.000 (T)
6	30.750	31.666	72.000 (T)	28.583	33.750
Mean	34.558	34.316	39.124	44.016	38.103
sd	3.4036	6.3921	16.140	13.833	12.986
p value 1		> 39.0	0.4043	0.1867	0.4044
p value 2	0.2862		0.2873	0.0458	0.2113

15 (T) - terminated at conclusion of experiment but showing symptoms of infection.

p value 1 refers to statistical significance when compared to pcDNA3.1 controls

20 p value 2 refers to statistical significance when compared to unvaccinated controls.

There was no significant difference in the survival times exhibited by the pcDNA3.1 and unvaccinated control groups. This is confirmed by their

very similar mean survival times of 34.558 hours (pcDNA3.1) and 34.316 hours (Unvaccinated).

5      **32 (ID-15)**

Mice immunised with the '32 (ID-15)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 and unvaccinated control groups. However, the '32 (ID-15)' group has two outlying mice one of which survived the term of the experiment despite showing symptoms. This group also exhibits a wide range of survival times.

15      **39 (ID-17)**

Mice immunised with the '39 (ID-17)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group but was not significant when compared with the pcDNA3.1 control group. The group has a considerably higher mean survival time of 44.016 hours than that determined for either of the control groups.

25      **57 (ID-40)**

Mice immunised with the '32 (ID-15)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 and unvaccinated control groups. However, the '32 (ID-15)' group has one outlying mouse which survived the term of the experiment despite showing symptoms.

30      **SURVIVAL DATA-SET B**

**Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 2 (Figure 5a)**

35

	Mean Survival Times (hours)		
	pcDNA	UnVacc	13(ID-72)
1	45.000	27.916	69.166
2	37.333	45.083	36.333
3	37.333	37.583	43.916
4	35.291	24.583	32.166
5	24.333	37.583	36.333
6	45.000	33.166	43.916
Mean	35.858	34.549	43.582
sd	7.4342	8.2567	14.917
p value 1		> 39.0	0.4679
p value 2	0.4679		0.1880

5 **p value 1** refers to statistical significance when compared to pcDNA3.1 controls.

**p value 2** refers to statistical significance when compared to unvaccinated controls.

10 There was no significant difference in the survival times exhibited by the pcDNA3.1 and unvaccinated control groups. This is confirmed by their very similar mean survival times of 35.858 hours (pcDNA3.1) and 34.166 hours (Unvaccinated).

### 15 **13 (ID-72)**

20 Mice immunised with the '13 (ID-72)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 and unvaccinated control groups. However, there is one outlying mouse which survived approximately 24 hours longer than the longest surviving mice in the pcDNA3.1 and unvaccinated control groups respectively. In addition, the time to first death in this group was prolonged when

compared to the pcDNA3.1 and unvaccinated control groups. The mean survival time of 43.582 hours is considerably higher than that determined for both control groups.

5

10

**Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 3 (Figure 5b)**

	Mean Survival Times (hours)		
	UnVacc	3-60(ID-65)	3-5(ID-66)
1	27.583	54.416	42.916
2	27.583	31.000	42.916
3	24.583	43.000	32.874
4	22.250	34.916	42.916
5	35.916	38.958	27.333
6	22.250	34.916	30.916
<b>Mean</b>	<b>27.583</b>	<b>40.458</b>	<b>37.791</b>
<b>sd</b>	<b>5.1691</b>	<b>8.9959</b>	<b>7.2860</b>
<b>p value</b>		<b>0.0098</b>	<b>0.0215</b>

15

**p value** refers to statistical significance when compared to unvaccinated controls.

20

**3-60 (ID-65)**

Mice immunised with the '3-60 (ID-65)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

5     **3-5 (ID-66)**

Mice immunised with the '3-5 (ID-66)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

### Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 4 (Figure 5c)

	Mean Survival Times (hours)			
	UnVacc	3-40(ID-67)	3-30(ID-68)	3-38(ID-69)
1	32.666	79.750	35.500	68.583
2	21.666	35.833	28.333	29.916
3	23.916	30.500	31.208	29.916
4	22.999	22.708	98.000 (T)	31.041
5	25.916	28.583	73.500	32.166
6	35.916	40.791	32.333	29.916
Mean	25.432	39.474	53.308	38.324
sd	4.3291	22.998	30.961	16.940
p value		0.1149	0.0463	0.1132

5

(T) - terminated at conclusion of experiment but showing symptoms of infection.

10

p value refers to statistical significance when compared to unvaccinated controls

#### 3-40 (ID-67)

15

Mice immunised with the '3-40 (ID-67)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there is one outlying mouse which survived approximately 43 hours longer than the longest surviving mice in the unvaccinated control group.

20

#### 3-30 (ID-68)

Mice immunised with the '3-30 (ID-68)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

**3-38 (ID-69)**

5 Mice immunised with the '2-19 (ID-73)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there was one outlying mouse which survived approximately 32 hours longer than the longest surviving mice in the unvaccinated control group. In addition, the time to first death was  
10 prolonged (by approximately 8 hours) when compared to the unvaccinated controls.

**Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 5 (Figure 5d)**

	Mean Survival Times (hours)				
	UnVacc	141(ID-70)	3-20(ID-71)	2-19(ID-73)	3-6(ID-74)
1	27.833	47.500	36.166	36.166	44.666
2	45.666	52.833	44.833	49.833	36.000
3	45.666	49.333	26.750	55.833	75.416
4	34.333	46.250	36.166	68.583	36.000
5	34.333	47.500	55.916	33.333	55.916
6	45.666	36.500	44.833	30.583	36.000
Mean	37.566	48.683	37.234	48.749	49.599
sd	7.8558	2.5672	8.4103	14.497	16.587
p value		0.0101	0.5000	0.2336	0.1854

15 **p value** - refers to statistical significance when compared to unvaccinated controls.

**141 (ID-70)**

20 Mice immunised with the '141 (ID-70)' DNA vaccine exhibited significantly longer survival times when compared with the unvaccinated control group.

**3-20 (ID-71)**

5 Mice immunised with the '3-20 (ID-71)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there is one outlying mouse which survived approximately 10 hours longer than the longest surviving mice in the unvaccinated control group.

**2-19 (ID-73)**

10 Mice immunised with the '2-19 (ID-73)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there are three outlying mouse which survived approximately 4, 10 and 23 hours longer than the longest surviving mice  
15 in the unvaccinated control group. This is reflected in the higher mean survival time of 48.749 hours and a much wider range of survival times.

**3-6 (ID-74)**

20 Mice immunised with the '3-6 (ID-74)' DNA vaccine did not show significantly longer survival times when compared with the unvaccinated control group. However, there are three outlying mouse which survived approximately 4, 10 and 23 hours longer than the longest surviving mice  
25 in the unvaccinated control group. This is reflected in the higher mean survival time of 49.599 hours and a much wider range of survival times.

**Statistical analysis of survival times - LEEP DNA immunisation and GBS challenge Trial 6 (Figure 5e)**

30

	Mean Survival Times (hours)			
	pcDNA3.1	UnVacc.	3-51(ID-75)	3-56 (ID-76)

1	33.541	36.000	36.333	46.583
2	36.750	29.999	30.291	29.833
3	36.750	32.749	32.000	40.166
4	36.750	44.500	52.333	46.583
5	29.000	28.333	72.000 (T)	46.583
6	30.750	31.666	40.499	---
<b>Mean</b>	<b>34.558</b>	<b>34.316</b>	<b>44.591</b>	<b>40.791</b>
<b>sd</b>	<b>3.4036</b>	<b>6.3921</b>	<b>16.615</b>	<b>7.9070</b>
<b>p value 1</b>		<b>&gt; 39.0</b>	<b>0.1876</b>	<b>0.0386</b>
<b>p value 2</b>	<b>0.2862</b>		<b>0.0867</b>	<b>0.0587</b>

(T) - terminated at conclusion of experiment but showing symptoms of infection.

5 **p value 1** refers to statistical significance when compared to pcDNA3.1 controls

**p value 2** refers to statistical significance when compared to unvaccinated controls.

10 There was no significant difference in the survival times exhibited by the pcDNA3.1 and unvaccinated control groups. This is confirmed by their very similar mean survival times of 34.558 hours (pcDNA3.1) and 34.316 hours (Unvaccinated).

15

### **3-51 (ID-75)**

Mice immunised with the '3-51 (ID-75)' DNA vaccine did not show significantly longer survival times when compared with the pcDNA3.1 control group but was relatively close to significant when compared with the unvaccinated control group. The '3-51' group has two outlying mouse one of which survived the term of the experiment despite developing symptoms. The mean survival time of 44.499 hours is considerably higher than that determined for both control groups and the group also demonstrates as a much wider range of survival times.

20

25

### 3-56 (ID-76)

5 Mice immunised with the '3-56 (ID-76)' DNA vaccine exhibited significantly longer survival times when compared with the pcDNA3.1 control group but were marginally not significant when compared with unvaccinated control group.

#### 10 **Example 3: Conservation and variability of candidate vaccine antigen genes among different isolates of Group B Streptococci**

15 An initial Southern blot analysis was carried out to determine cross-serotype conservation of novel Group B Streptococcal genes isolated using the LEEP system. Analysing the serotype distribution of a target gene will also determine their potential use as antigen components in a GBS vaccine. The Group B Streptococcal strains whose DNA was analysed as part of this study are listed in **APPENDIX II**.

#### **Amplification and labelling of specific target genes as DNA probes for Southern blot analysis.**

20 Oligonucleotide primers were designed for each individual gene of interest derived using the LEEP system. Primers were designed to target the whole of the gene being investigated (All primers are listed in **APPENDIX III**). Specific gene targets were amplified by PCR using Vent DNA polymerase (NEB) according to the manufacturers instructions. Typical reactions were carried out in a 100 µl volume containing 50 ng of  
25 GBS template DNA, a one tenth volume of enzyme reaction buffer, 1 µM of each primer, 250 µM of each dNTP and 2 units of Vent DNA polymerase. A typical reaction contained an initial 2 minute denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at the appropriate melting temperature for 30 seconds, and extension at 72°C for 1 minute (1 minute per kilobase of DNA  
30 being amplified). The annealing temperature was determined by the lower melting temperature of the two oligonucleotide primers. The reaction was concluded with a final extension period of 10 minutes at 72°C.

All PCR amplified products were extracted once with phenol chloroform (2:1:1) and once with chloroform (1:1) and ethanol precipitated. Specific DNA fragments were isolated from agarose gels using the QIAquick Gel Extraction Kit (Qiagen). For use as DNA probes, purified amplified gene DNA fragments were labelled with digoxigenin using the DIG Nucleic Acid Labelling Kit (Boehringer Mannheim) according to the manufacturer's instructions.

#### **Southern blot hybridisation analysis of Group B Streptococcal genomic DNA**

Genomic DNA had previously been isolated from all strains of Group B Streptococci which were investigated for conservation of LEEP-derived gene targets. Appropriate DNA concentrations were digested using either *Hin* DIII, *Eco* RI or *Bgl* II restriction enzymes (NEB) according to manufacturer instructions and analysed by agarose gel electrophoresis. Following agarose gel electrophoresis of DNA samples, the gel was denatured in 0.25M HCl for 20 minutes and DNA was transferred onto Hybond™ N<sup>+</sup> membrane (Amersham) by overnight capillary blotting. The method is essentially as described in Sambrook *et al.* (1989) using Whatman 3MM wicks on a platform over a reservoir of 0.4M NaOH. After transfer, the filter was washed briefly in 2x SSC and stored at 4 °C in Saran wrap (Dow chemical company).

Filters were prehybridised, hybridised with the digoxigenin labelled DNA probes and washed using conditions recommended by Boehringer Mannheim when using their DIG Nucleic Acid Detection Kit. Filters were prehybridised at 68°C for one hour in hybridisation buffer (1% w/v supplied blocking reagent, 5x SSC, 0.1% v/v N-lauryl sarcosine, 0.02% v/v sodium dodecyl sulphate[SDS]). The digoxigenin labelled DNA probe was denatured at 99.9°C for 10 minutes before being added to the hybridisation buffer. Hybridisation was allowed to proceed overnight in a rotating Hybaid tube in a Hybaid Mini-hybridisation oven. Unbound probe was removed by washing the filter twice with 2x SSC- 0.1% SDS for 5 minutes at room temperature. For increased stringency filters were then washed twice with 0.1x SSC-0.1% SDS for 15 minutes at 68°C. The DIG Nucleic Acid Detection Kit (Boehringer Mannheim) was used to immunologically detect specifically bound digoxigenin labelled DNA probes.

### Results of Southern blot analysis

All genomic digests and their corresponding Southern blots followed an identical lane order as described in Table I.

5 **Table I**

1 kb molecular Weight Marker	515	A909	SB35	H36B	18RS21	1954/92
	Ia	Ia	Ib	Ib	II	II

118/158	97/0057	BM110	BS30	M781	97/0099	3139
II	II	III	III	III	III	IV

1169-NT	GBS 6	7271	JM9	Group A <i>Streptococcus</i> <i>pneumoniae</i>
V	VI	VII	VIII	—

10 For comparative purposes, it was decided to analyse the serotype distribution of the GBS *rib* gene, which encodes the known protective immunogen Rib. Rib has previously been shown to be present in serotype III and some strains of serotype II but not in serotypes Ia or Ib (Stalhammar-Carlemalm *et al.*, 1993). Confirmation of this pattern would not only give increased confidence in interpreting subsequent results, it would also determine if a *rib* gene homologue was present in the remaining GBS

serotypes being investigated here. Primers designed for the amplification of *rib* and its subsequent cloning into pcDNA3.1 (Appendix I), were used to generate a *rib* gene probe for Southern blot analysis.

## 5 Southern blot analysis - *rib* (Figure 6)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

10

Genomic DNA from each strain was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled *rib* gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

15

### Comment

The Southern blot analysis described in Figure 7 indicates that the *rib* gene is not conserved across all GBS serotypes. *rib* appears to be absent from all serotype Ia and Ib strains (lanes 2 to 5) and from strains 118/158 and 97/0057 of serotype II (lanes 8 and 9). However, *rib* would appear to present in strains 18RS21 and 1954/92 of serotype II (lanes 6 and 7) and in all strains of serotype III (lanes 10 to 13). This is in agreement with previously published data (Stalhammar-Carlemalm *et al.*, 1993). *rib* would also appear to be present in strains representing serotypes VII and VII (lanes 17 and 18) but was absent from strains representing serotypes IV, V and V (lanes 14 to 16) as well as the control strains (lanes 19 and 20). The *rib* gene probe did hybridise with lower intensity to genomic DNA fragments from strains representing serotypes Ia, Ib, IV, VI, VII and serotype II strains 118/158 and 97/0057. This may indicate the presence of a gene in these strains with a lower level of homology to *rib*. These hybridising DNA fragments may contain a homologue of the GBS *bca* gene encoding the Ca protein antigen which has been shown to be closely homologous to the Rib protein (Wastfelt *et al.*, 1996). If this is the case, it would be in agreement with previous work which showed all strains of serotypes Ia, Ib, II and III to be positive for one the two proteins (Stalhammar-Carlemalm *et al.*, 1993). However, the apparent

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variable distribution of the *rib* gene amongst different GBS serotypes, makes it a less than ideal candidate for use in a GBS vaccine that is cross-protective against all serotypes.

5     **Southern blot analysis - 4 (ID-1) (photograph 7)**

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

10     Genomic DNA from each strain was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled 4 (ID-1) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

15

Comment

The Southern blot analysis described in Figure 7 indicates that gene 4 (ID-1) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Hin* DIII-digested genomic DNA fragment of approximately 3.5 kb in DNA digests from all GBS representatives. but was absent from both the control strains (lanes 19 and 20).

20

**Southern blot analysis - 5 (ID-2) (Figure 8)**

25

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

30     Genomic DNA from each strain was digested completely with *Eco* RI (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled 5 (ID-2) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

Comment

The Southern blot analysis described in Figure 7 indicates that gene 4 (ID-1) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Eco* RI-digested genomic DNA fragment of approximately 6.2 kb in DNA digests from all GBS representatives, but was absent from both the control strains (lanes 19 and 20).

#### Southern blot analysis - 15 (ID-7) (Figure 9)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Genomic DNA from each strain was digested completely with *Eco* RI (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled 15 (ID-7) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

#### Comment

The Southern blot analysis described in Figure 7 indicates that gene 15 (ID-7) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Eco* RI-digested genomic DNA fragment of approximately 6.2 kb in DNA digests from all GBS representatives, but was absent from both the control strains (lanes 19 and 20). The gene probe hybridised specifically with *Eco* RI -digested DNA fragments ranging from approximately 3.5 kb to 5.2 kb in size.

#### Southern blot analysis - 17 (ID-8) (Figure 10)

#### Figure 5

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Genomic DNA from each strain was digested completely with *Hin* DIII (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled

17 (ID-8) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

#### Comment

5 The Southern blot analysis described in Figure 7 indicates that gene 17 (ID-8) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Hin* DIII-digested genomic DNA fragment of approximately 2.3 kb in DNA digests from all GBS representatives. but was absent from both the control strains (lanes 19 and 20).

10

#### **Southern blot analysis - 22 (ID-10) (Figure 11)**

#### **Figure 6**

15 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Genomic DNA from each strain was digested completely with *Bgl* II (NEB) and electrophoresed at 40 Volts for 6 hours in 0.8% agarose, transferred onto Hybond N<sup>+</sup> (Amersham) membrane by Southern blot and hybridised with the digoxigenin-labelled 22 (ID-10) gene probe. Specifically bound DNA probe was identified using the DIG Nucleic Acid Detection Kit (Boehringer Mannheim).

20

#### Comment

25 The Southern blot analysis described in Figure 7 indicates that gene 22 (ID-10) is conserved across all GBS serotypes. The gene probe hybridised specifically to a *Bgl* II-digested genomic DNA fragment of approximately 3.1 kb in DNA digests from all GBS representatives except serotype Ib strain H36B, where the gene probe hybridised specifically to a *Bgl* II-digested genomic DNA fragment. Gene 22 (ID-10) was absent from both the control strains (lanes 19 and 20).

30

#### **Conclusion**

The Southern blot analyses described here, represents a preliminary investigation into the conservation level of LEEP-derived genes amongst different GBS serotypes. Initial results indicate that the genes 4 (ID-1), 5 (ID-2), 15 (ID-7), 17(ID-8) and 22

(ID-10) are present in all GBS serotypes and thus represent potential candidate genes for use in a GBS vaccine. Similar analyses are being currently carried out for each of the genes contained in this patent.

**APPENDIX I****ID-8 (17)**

Forward Primer

5' - cgggatccgccaccatgACCACTTCTCAAGCTGTTTTCAGC - 3'

Reverse Primer

5' - ttgcggccgcACGATTATCAACAAAGTTCTG - 3'**ID-9 (18)**

10 Forward Primer

5' - cgggatccgccaccatgGCTACTCATATTGGAAGTTACCAGC - 3'

Reverse Primer

5' - ttgcggccgcAGGGTTTATTTGTTGAAGTGTCTTG - 3'15 **ID-10 (22)**

Forward Primer

5' - cgggatccgccaccatgTATCTATATCATTTACCAATGCCC - 3'

Reverse Primer

5' - ttgcggccgcTTTATGTATAGAAACAGCAGTCCC - 3'

20

**ID-13 (28)**

Forward Primer

5' - cgggatccgccaccatgAAAGGAAGAACAACCTATTCGTTTAG - 3'

Reverse Primer

25 5' - ttgcggccgcAAGAGCAAATTTTCGTATCTCCTC - 3'**ID-15 (32)**

Forward Primer

5' - cgggatccgccaccATGATTGTTGGACACGGAATTG - 3'

30 Reverse Primer

5' - ttgcggccgcTTTTTCTTCCTCCAAAATAACACTAGC - 3'**ID-17 (39)**

Forward Primer

5' - cggatccgccaccatgGCGACTAAAGAGTTAGGTGTTAG -3'

Reverse Primer

5' - ttgcggccgcTATAGTTTTAGTTTCAACTTGTCTAGATG -3'

5 ID-25 (20)

Forward Primer

5' - cgggatccaccatgTATACGAGTTTACAACCAAATCATG -3'

Reverse Primer

5' - ttgcggccgcGTCAGCTCGTACTGTTTTTTTAGC -3'

10

ID-37 (51)

Forward Primer

5' - cggatccgccaccatgTGTCAAATGAATAGTGAACATAAAAG -3'

Reverse Primer

15 5' - ttgcggccgcCTCAAATAATTTACCTCCAATTTCG -3'

ID-40 (51)

Forward Primer

5' - cggatccgccaccatgGCTCCATTCGAATTAAAGATTC -3'

20 Reverse Primer

5' - ttgcggccgcTGATTTACCAGTTTGGAAGAGTTC -3'

ID-42 (70)

Forward Primer

25 5' - cggatccgccaccATGAATACTATTTATAATACATTGAGAACAG -3'

Reverse Primer

5' - ttgcggccgcTTCTTTGTTCCAACCTTTCTGG -3'

ID-47 (86)

30 Forward Primer

5' - cggatccgccaccATGATAGAGTGGATTCAAACACATTTAC -3'

Reverse Primer

5' - ttgcggccgcTTTATGACTCAAGCGACGTGTTA -3'

ID-48 94

Forward Primer

5' - cggatccgccaccATGGAGTTAGTAATTAGAGATATTCGTAAG

Reverse Primer

5 5' - ttgcggccgcCTTGTCATATTCATCTCCCTTCAACID-67 (3-40)

Forward Primer

5' - cggatccgccaccatgGCTAGTTTTGTCATGAATCATAATGAC -3'

10 Reverse Primer

5' - ttgcggccgcGTTATTTGCTCGTTGTTTAGCTAAATC -3'ID-68 (3-30)

Forward Primer

15 5' - cggatccgccaccatgGCTCTTAGTTTTTTTATGGTTTCAGTTCAAGC -3'

Reverse Primer

5' - ttgcggccgcGAAGGCACCGCCACCTCC -3'ID-69 (3-38)

20 Forward Primer

5' - cggatccgccaccatgGGTGAAACCCAAGATACCAATCAAGC -3'

Reverse Primer

5' - ttgcggccgcAACACCTGGTGGGCGTTTGG -3'25 ID-70 (141)

Forward Primer

5' - cggatccgccaccATGGCTGGGAATCGTAATAACG -3'

Reverse Primer

30 5' - ttgcggccgcAGCCGTCTCTAAAACAGGCTTG -3'ID-71 (3-20)

Forward Primer

5' - cggatccgccaccatgCTTCCAACGCAGCCGCAAAAC -3'

Reverse Primer

5' - ttgcggccgcATTAGTGTTATTTCTCCTGTTGCATAATCC -3'

ID-72 (13)

Forward Primer

5' - cgggatccaccatgTACACGCATATTGTTGAAAAAAG -3'

Reverse Primer

5' - ttgcggccgcAAATAATTTCTTTTGGTTGTTTG -3'

ID-73 (2-19)

10 Forward Primer

5' - cggatccgccaccatgAGTAATCAAGAAGTTTCAGCAAGC -3'

Reverse Primer

5' - ttgcggccgcCCATTGTGGAATATCAGCTGAAG -3'

15 ID-74 (3-6)

Forward Primer

5' - cggatccgccaccatgGTGCAGGCAGTGGTACCGCT -3'

Reverse Primer

20 5' - ttgcggccgcGCGCATTGTAACAAATTCCTCAG -3'

ID-75 (3-51)

Forward Primer

5' - cgggatccaccatgGCTGCCGAGAAGGATAAAG -3'

25 Reverse Primer

5' - ttgcggccgcATTATTTAGCTGCTTTTTTAATGG -3'

ID-76 (3-56)

Forward Primer

30 5' - cgggatccaccatgTGTCAGGTTGTTTATGCAAGTTTTC -3'

Reverse Primer

5' - ttgcggccgcTTTACTAATTGATAAAGAGCAACTTCG -3'

*rib* (control)

Forward primer

5' - ggggtaccggccaccATGGCTGAAGTAATTCAGGAAGT -3'

Reverse primer

5' - cggaattccgTTAATCCTCTTTTTTTCTTAGAAACAGAT

**APPENDIX II**

Listed below are the details (serotype and strain designation) of Group B *Streptococcus* strains whose DNA was analysed for gene conservation

5

SEROTYPE	STRAIN
Ia	515
Ia	A909
10 Ib	SB35
Ib	H36B
II	18RS21
II	1954/92
II	118/158
15 II	97/0057
III	BM110
III	BS30
III	M781
III	97/0099
20 IV	3139
V	1169/NT
VI	GBS VI
VII	7271
VIII	JM9

25

A group A Streptococcal strain (serotype M1, strain NCTC8198) and *Streptococcus pneumoniae* (serotype 14) were also included in the analysis for control purposes.

**APPENDIX III**ID-1 (4)

forward primer

5 5' - atggaaaaaataacttggaataattac -3'

reverse primer

5' - ctatttgttttagcgatgtctttatc -3'

ID-2 (5)

10 forward primer

5' - atgtcaaaacaaaagtaacggcaac -3'

reverse primer

5' - ttatttatggccaataaccataagttaattg

15 ID-6 (9)

forward primer

5' - atgaaaaagttttttctcatggctatg -3'

reverse primer

5' - ttacttcaactgttgatagagcacttc - 3'

20

ID-7 (15)

forward primer

5' - ttgttcaattttataggtttagaacttgg -3'

reverse primer

25 5' - ttaattttcattgcgtctcaaacc -3'

ID-8 (17)

forward primer

5' - atgacaaaaaacttattattgctatattag -3'

30 reverse primer

5' - ttaacgattatcaacaaagtctgtac -3'

ID-10 (22)

forward primer

5' - atgatacgccagttttaagagaa -3'

reverse primer

5' - ttatttatgtatagaaacagcagtc -3'

## 5 References

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Kurar, E. and Splitter, G.A. (1997) Nucleic acid vaccination of *Brucella abortus* ribosomal L7/L12 gene elicits immune response. *Vaccine*, **15**, 1851-57.

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Larsson, C., Stalhammar-Carlemalm, M., and Lindahl, G. 1999. Protection against experimental infection with Group B Streptococcus by immunization with a bivalent protein vaccine. *Vaccine*. **17**:454-458

Stalhammar-Carlemalm, M., Stenberg, L., and Lindahl, G. 1993. Protein Rib: a novel Group B Streptococcal protein that confers protective immunity and is expressed by most strains causing invasive infections: *J. Exp. Med.* **177**: 1593-1603

Wastfelt, M., Stalhammar-Carlemalm, M., (1996) Identification of a family of Streptococcal surface proteins with extremely repetitive structure. *J. Biol. Chem.* **271**: 18892-18897.

Zhang, D., Yang, X., Berry, J. Shen, C., McClarty, G. and Brunham, R.C. (1997) DNA vaccination with the major outer-membrane protein genes induces acquired immunity to *Chlamydia trachomatis* (mouse pneumonitis) infection. *Infection and Immunity*, **176**, 1035-40.

## Claims:

1. A *Group B Streptococcus* protein having a sequence selected from those described in fig 1, or fragments or derivatives thereof.
2. A *Group B Streptococcus* polypeptide or peptide having a sequence selected from those described in fig 2, or fragments or derivatives thereof.
3. Derivatives or variants of the proteins, polypeptides, and peptides as claimed in claims 1 and 2 which show at least 50% identity to those proteins, polypeptides and peptides claimed in claims 1 and 2.
4. A nucleic molecule comprising or consisting of a sequence which is:
  - (i) any of the DNA sequences set out in figure 1 and figure 2 herein or their RNA equivalents;
  - (ii) a sequence which is complementary to any of the sequences of (i);
  - (iii) a sequence which codes for the same protein or polypeptide, as those sequences of (i) or (ii);
  - (iv) a sequence which shows substantial identity with any of those of (i), (ii) and (iii); or
  - (v) a sequence which codes for a derivative, or fragment of a nucleic acid molecule shown in figure 1 or figure 2.
5. A vector comprising DNA encoding for the expression of any one or more proteins, polypeptides, peptides, fragments or derivatives thereof, as claimed in claims 1 to 3.
6. A vector as claimed in claim 5 further comprising DNA encoding any one or more of the following: promoters, enhancers, signal sequences, leader sequences,

translation start and stop signals, DNA stability controlling regions, or a fusion partner.

5 7. The use of a vector as claimed in claims 5 and 6 in the transformation or transfection of a prokaryotic or eukaryotic host.

8. A host cell suitable for the transformation of vector as claimed in claims 5 and 6.

10 9. An antibody, an affibody, or a derivative thereof which binds to one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, as claimed in any one of claims 1 to 3.

15 10. An immunogenic composition comprising one or more of the proteins, polypeptides, peptides, fragments or derivatives thereof, or nucleic acid sequences as claimed in any one or more of claims 1-3 and claim 4.

11. An immunogenic composition as claimed in claim 10 which is a vaccine.

20 12. Use of an immunogenic composition as claimed in claim 10 in the preparation of a medicament for the treatment or prophylaxis of *Group B Streptococcus* infection.

25 13. A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one antibody, affibody, or a derivative thereof, as described herein.

30 14. A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one protein, polypeptide, peptide, fragments or derivatives as described herein.

15. A method of detection of *Group B Streptococcus* which comprises the step of bringing into contact a sample to be tested with at least one nucleic acid molecule as described herein.

5

16. A kit for the detection of *Group B Streptococcus* comprising at least one antibody, affibody, or derivatives thereof as claimed in claim 9.

10 17. A kit for the detection of *Group B Streptococcus* comprising at least one *Group B Streptococcus* protein, polypeptide, peptide, fragment or derivative thereof as claimed in claims 1 to 3.

18. A kit for the detection of *Group B Streptococcus* comprising at least one nucleic acid molecule as claimed in claim 4.

15

19. A method of screening for DNA encoding bacterial cell envelope associated or surface antigens in gram positive bacteria comprising the steps of:

- 20
- combining a reporter vector including the nucleotide sequence encoding the mature form of the staphylococcus nuclease gene and an upstream promoter region with DNA from a gram positive bacteria.
  - transforming the resultant vector into *Lactococcus lactis* cells.
  - assaying for the secretion of staphylococcus nuclease protein in the transformed cells.

25 20. A method as claimed in claim 19 wherein the reporter vector is one of the pTREP1-*nuc* vectors shown in figure 4.

30 21. A method as claimed in claim 19 or claim 20 wherein the gram positive bacteria is *Group B Streptococcus*, *Streptococcus Pneumoniae*, *Staphylococcus aureus* or pathogenic group A streptococci.

22. A vector as shown in figure 4 for use in screening for DNA encoding bacterial cell envelope associated or secreted antigens in gram positive bacteria.
- 5 23. A method of determining whether a protein, polypeptide, peptide, fragment or derivative thereof as claimed in claims 1 to 3 represents a potential anti-microbial target which comprises inactivating said protein and determining whether *Group B Streptococcus* is still viable.

ID-1

## FIG. 1

Clone 4

ATGGAAAAAATACTTGGAAAAAATTACTTGTTAGTACTGCTGCTCTTTCAGTAGT  
TGCAGGAGGAGCAATTGCTGCTACTCACTCTAACTCAGTTGATGCTGCTTCAAAAA  
AACTATCAAACCTTGGGTCCCAACAGATTCAAAAGCGTCTTATAAAGCAATTGTT  
AAAAAATTCGAGAAGGAAAACAAAGGCGTTACTGTAAAAATGATTGAGTCTAATG  
ACTCCAAAGCTCAAGAAAACGTAAAAAAGACCCAAGCAAGGCAGCCGATGTATT  
CTCACTTCCACATGACCAACTTGGTCAATTAGTAGAATCTGGTGTTATCCAAGAAA  
TTCCAGAGCAATACTCAAAAGAAATTGCTAAAAACGACACTAAACAATCACTTAC  
TGGTGCACAATATAAAGGGAAAACTTATGCATTCCCATTTGGTATTGAATCTCAAG  
TTCTTTATTATAATAAAACAAAGTTAACTGCTGACGACGTTAAATCATACGAAACA  
ATTACAAGCAAAGGGGAAATTCGGTCAACAGCTTAAAGCAGCTAACTCATATGTAA  
CAGGTCCTCTTTTCTTTCTGTAGGCGACACTTTATTTGGTAAATCTGGTGAAGATG  
CTAAAGGCACTAACTGGGGTAATGAAGCAGGTGTTTCTGTCCTTAAATGGATTGCA  
GATCAAAAGAAAAATGATGGTTTTGTCAACTTGACAGCTGAAAATACAATGTCTAA  
ATTTGGCGATGGTTCTGTTTCATGCTTTTGAAGTGGAACCATGGGATTACGACGCTG  
CTAAAAAAGCTGTCGGTGAAGATAAAATCGGTGTTGCTGTTTACCCAACAATGAAA  
ATCGGTGACAAAGAAGTTCAACAAAAAGCATTCTTGGGCGTTAACTTTATGCCGT  
TAACCAAGCACCTGCTGGTTCAAACACTAAACGAATCTCAGCTAGCTACAACTCG  
CTGCATATCTAACTAATGCTGAAAGTCAAAAAATTCAATTCGAAAAACGTCATATC  
GTTCTGCTAACTCATCAATTCAATCTTCTGATAGCGTCCAAAAAGATGAACTTGC  
AAAAGCAGTTATCGAAATGGGTAGCTCAGATAAATATACAACGGTTATGCCTAAG  
TTGAGTCAAATGTCAACATTCTGGACAGAAAGTGCTGCTATTCTTAGCGATACTTA  
CAGTGGTAAAATCAAATCTAGCGATTACCTTAAACGTCTAAAACAATTCGATAAAG  
ACATCGCTAAAACAAAATAG

MEKNTWKLLVSTAALSVVAGGALAATHSNSVDAASKKTIKLWVPTDSKASYKAIVK  
KFEKENKGVTVKMIESNDSKAQENVKKDPSKAADVFSPLHDQLGQLVESGVIQEIQ  
YSKEIAKNDTKQSLTGAQYKGKTYAFPGIESQVLYYNKTKLTADDVKSYETITSK  
FGQQLKAANSYVTGPLFLSVGDTLFGKSGEDAKGTNWGNEAGVSVLKWIADQKKND  
GFVNLTAEENTMSKFGDGSVHAFESGPWDYDAAKKAVGEDKIGVAVYPTMKIGDKEV  
QQKAFLGVKLYAVNQAPAGSNTKRISASYKLAAYLTNAESQKIQFEKRHIVPANSSIQS  
SDSVQKDELAKAVIEMGSSDKYTTVMPKLSQMSTFWTESAAILSDTYSKIKSSDY  
LKRLKQFDKDIATKZ

ID-2

Clone 5

ATGTCAAAAACAAAAAGTAACGGCAACTTTGTTGTTATCCACTTTAGTCTTATCGCT  
ATCATCACCTTTAGTGACCTTAGCAGAAACTATTAATCCAGAAACAAGCCTGACAA  
TGGCAACAGCATCAACAGAAAGTTCTTCTGAAGCAGAGAAACAGGAAAAAACACA  
ACCTACAGATTGAGAACTGCTTCACCTTCAGCCGAAGGAAGTATCTCAACAGAA  
AAAACAGAGATTGGTACGACAGAGACATCATCAAGCAATGAATCATCATCAAGTT  
CATCACATCAATCTTCTTCCAACGAAGATGCTAAAACATCTGATTCTGCTTCAACA  
GCATCTACTCCTAGCACTAATACTACAAACAGTAGTCAAGCAGACAGTAAGCCAG  
GTCAATCAACAAAGACTGAATTAACCTGAGCCTACCTTACCATTAGTAGAGCCT  
AAAATAACTCCCGCTCCGTCTCAGATAGAAAGTGTTTCAACAAATCAGAATGCTTC  
TGTTCTGCTTTATCCTTTGATGATAACTTATTATCAACACCGATTTCACCAGTGAC  
AGCAACGCCATTCTACGTAGAACACTGGTCTGGTCAGGATGCCTACTCTCACTATT  
TATTGTCACATCGTTACGGTATCAAAGCTGAACAATTAGATGGGTACTTAAAATCT  
TTAGGGATTCAATATGATTCTAATCGTATCAATGGTGCTAAGTTATTACAATGGGA  
AAAAGATAGTGGTTTAGATGTCCGTGCTATTGTAGCTATTGCTGTCCTTGAAAGTTC  
ATTGGGAACTCAAGGAGTGGCTAAAATGCCAGGTGCTAATATGTTTGGTTATGGTG  
CCTTTGATCATGACTCTAGCCATGCTAGTGCTTATAATGATGAAGAAGCAATTATG  
TTGTTGACAAAAAATACAATTATTAACAAACAACACTCTAGCTTTGAAATCCAAGA  
TTTGAAAGCACAGAAATTATCTTCTGGACAACCTAATACAGTTACTGAGGGTGGTG  
TTTATTATACAGATAACTCTGGAACCTGGTAAACGTCGTGCCAGATTATGGAAGAT  
TTAGACCGCTGGATTGATCAACATGGAGGGACACCAGAAATTCCTGCTGCCTTGAA  
AGCTTTATCGACAGCAAGTTTAGCAGATTTACCAAGTGGTTTTAGCTTATCAACAG  
CGGTTAACACAGCTAGCTATATTGCATCAACTTATCCATGGGGTGAATGTACATGG  
TATGTCTTTAACCGCGCTAAAGAGTTAGGTTATACATTTGATCCATTTATGGGTAAT  
GGTGGAGATTGGCAACATAAGGCTGGCTTTGAAACAACACATTCACCAAAAAGTAG  
GCTATGCTGTATCATTTTCACCAGGACAAGCTGGTGCTGATGGCACTTACGGTCAC  
GTAGCTATTGTTGAAGAAGTTAAAAAAGATGGTTCAGTTCTCATTTCAGAATCTAA  
TGCAATGGGACGTGGTATTGTCTCTTACCGTACTTTTAGTTTCAGCACAAGCTGCAC  
AATTAACCTTATGGTATTGGCCATAAATAA

MSKQKVTATLLLSTLVLSLSSPLVTLAETINPETSMTASTESSSEAEKQEKTOPTDS  
ETASPSAEGSISTEKTEIGTTETSSSSNESSSSSSHQSSSNEDAKTSDSASTASTPSTNTNS  
SQADSKPGQSTKTELKPEPTLPLVEPKITPAPSQIESVQTNQNASVPALSFDDNLLSTPIS  
PVTATPFYVEHWSGQDAYSHYLLSHRYGIKAEQLDGYLKSGLGIQYDSNRINGAKLLQ  
WEKDSGLDVRAIVAIAVLESSLGTQGVAKMPGANMFGYGAFDHDSSHASAYNDEEAI  
MLLTKNTIKNNNSSFEIQDLKAQKLSSGQLNTVTEGGVYYTDNSGTGKRRAQIMEDL  
DRWIDQHGGTPEIPAALKALSTASLADLPSGFSLSTAVNTASYIASTYPWGECTWYVF  
NRAKELGYTFDPFMGNGGDWQHKAGFETTHSPKVGAVVSFSPGQAGADGTYGHVAI  
VEEVKKDGSVLISESNAMGRGIVSYRTFSSAQAAQLTYGIGHKZ

FIG. 1 CONT'D

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ID-3

Clone 6

GTGCATATGTTACAAAACATTGGACAAACAGGCATTCAAGCAACTCGAATTGCTTT  
AGGTTGTATGAGAATGAGTGACTTGAAAGGAAAACAAGCTGAAGAAGTAGTTGGA  
ACAGCATTAGATTTGGGTATTATAAATAATAAAGTGCAAGAAAGTGTCTCTGGCGT  
CAAAGTGACTAAATCATTGTGTTATCAAGAACAAGAAATTGCTTCTTTTCAAGAGA  
TTAATCAGATGACTTTCGTGAAGAACATGCGGACCATGACTTATGATGTCATGTTT  
GATCCTTTAGTTCTTCTTTTATAGGTGCCTCCTACGTATTAACATTGGCTATGGGA  
GCTTTTATGATTTCAAAAGGTCAAGTTACTGTTGGTGACTTGGTAACATTTGTGACG  
TATTTAGATATGTTGGTATGGCCCTTGATGGCGATTGGTTTCTTGTTCAATATGGTA  
CAGCGTGGTAGTGTTTCTTATAACCGTATTAATAGTCTACTTGAGCAAGAATCGGA  
TATAACTGATCCTTTAAATCCTATCAAACCTGTTGTCAATGGAACATTAAGATA  
TGATATTGATTTCTTTAGATACGACAATGAGGAAACCTTAGCCGATATTCATTTTAC  
CTTAGAAAAAGGTCAAACCTTAGGTTTGGTAGGTCAAACGGGATCAGGGAAGACA  
AGTCTTATTAAGTTATTGCTACGTGAACATGATGTGACTCAGGGGAAAATTACTTT  
AAATAAACATGATATACGTGATTATCGATTGTCTGAGTTACGTCAACTAATCGGTT  
ATGTTCTCAAGATCAGTTTTTTATTTGCTACCAGTATTTTAGAAAAATGTTGCTTTG  
GAAATCCAACCTCTATCTAATGCTGTCAAAGAAGCAACTAAATTGGCACATGTT  
TACGATGACATTGAACAGATGCCAGCAGGATTTGAGACTCTAATTGGAGAAAAAG  
GAGTCTCATTATCTGGTGGACAAAAACAAAGGATTGCGATGAGTCGTGCCATGATT  
TTAGATCCAGATATTCTTATTTTGGATGATTCTCTATCAGCAGTGGACGCTAAAACG  
GAACATGCTATTGTTGAGAATCTTAAACGAATCGTCAAGGGAAATCGACTATTA  
TTTCAGCACATCGTTTATCAGCTGTTGTGCACGCAGACCTTATCTTAGTTATGCGAG  
ACGGCAGAGTCATTGAGCGAGGTCAACATCAAGAGTTGCTAAATAAAGGTGGTTG  
GTATGCTGAAACGTATGCCTCACAGCAATTAGAAATGGAGGAAGCATTGTGAA  
GTCTAA

MHMLQNIGQTGIQATRIALGCMRMSDLKKGKQAEVVGTAALDLGIINNKVQESVSGVK  
VTKSLCYQEIEASFQEQINQMTFVKNMRTMTYDVMFDPLVLLFIGASYVLTAMGAF  
MISKGQVTVGDLVTFVTYLDMLVWPLMAIGFLNMVQRGSVSYNRINSLEQESDITD  
PLNPIKPVVNGTLRYDIDFFRYDNEETLADIHFTLEKGQTLGLVGQTGSGKTSLIKLLR  
EHDVTQGKITLNKHDIRDYRLSELRLIGYVPQDQFLFATSILENVRFGNPTLSINAVKE  
ATKLAHVYDDIEQMPAGFETLIGEKGVSLSGGQKQRIAMSRAMILDPDILDDSLSAV  
DAKTEHAIVENLKTNRQKSTIISAHRLSAVVHADLILVMRDGRVIERGQHQELLNKG  
GWYAETYASQQLEMEEAFDEVZ

ID-4

Clone 6b

TTGATGAAGTCTAATCAATGGCAAGTCTTTAAGAGATTAATCTCCTATTTACGCCCT  
TATAAATGGTTTACAGTATTAGCTCTATCTCTTATTGTTGACGACTGTTGTAA

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FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

AATATTATTCTTTAATTGCTTCACATTTTATTGATCACTATCTGACAAATGTTAAT  
CAAACAGCAGTTCTTATTTTAGTGGGATATTATTCAATGTATGTCTTGCAGACCTTA  
ATTCAATATTTTGGGAATCTCTTTTTTGCGCGTGTCTTATAGTATTGTTAGAGAT  
ATTCGTAGAGATGCTTTTGCTAATATGGAAAGGCTAGGCATGTCTTATTTTGATAG  
GACACCGGCAGGATCTATTGTGTCACGTATTACTAATGATACTGAAGCAATATCTG  
ATATGTTTTTCGGGTATTTTATCAAGTTTTATCTCGGCGATATTTATTTTACAGTTAC  
TCTGTACACTATGTTGATGCTAGACATTAACTAACAGGACTCGTCGCTCTTTTGTT  
ACCTGTTATCTTTATATTAGTGAATGTCTATCGGAAAAAATCAGTCACTGTCATTGC  
TAAAACGAGAAGTTTACTTAGTGATATCAACAGTAAATTATCAGGAAGTATTGAAG  
GAATTCGCATTGTACAGGCTTTTGGTCAAGAAGAGCGCTTGAAGACTGAATTTGAG  
GAAATTAACAAAGAGCATGTTGTGTATGCCAATCGTTCTATGGCTCTTGATAGTCT  
CTTCTTAAGACCGGCGATGTCTCTTTTAACTCCTAGCATATGCTGTTCTTATGTC  
TTATTTTGGATTTACAGGAGTTAAAGGAGGTCTTACGGCAGGATTAATGTATGCTT  
TTATTCAGTACGTTAATCGTCTATTTGACCCTTAATTGAAGTAACGCAAAATTTT  
CAACCTTACAAACATCAATGGTATCAGCAGGGCGTGTGTTTGATCTGATTGAT  
GAAACAGGTTTTGAACCAAGCCAAAAAATACAGAAGCT

MKSNQWQVFKRLISYLRPYKWFTVLALSLLLLTTVVKNIPLIASHFIDHYLTNVNQTA  
VLILVGYYSMYVLQTLIQYFGNLFARVSYSIVRDIRRDAFANMERLGMSYFDRTPAG  
SIVSRITNDTEAISDMFSGILSSFISAIFITVTLYTMLMLDIKLTGLVALLLPVIFILVNVY  
RKKSVTVIAKTRSLSDINSKLSGSIEGIRIVQAFGQEERLKTEFEEINKEHV VYANRSM  
ALDSLFLRPAMSLKLLAYAVLMSYFGFTGVKGGTLTAGL MYAFIQYVNRLFDPLIEVT  
QNFSTLQTSMV SAGR VFDLIDETGFEPSQKNTEA

ID-5

Clone 7

ATGAAAAGAAAAGACTTATTTGGTGATAAACA AACTCAATACACGAT  
TAGAAAGTTAAGTGTTGGAGTAGCTTCAGTTGCAACAGGGGTATGTA  
TTTTTCTTCATAGTCCACAGGTATTTGCTGAAGAAGTAAGTGTTTCTC  
CTGCAACTACAGCGATTGCAAAGTCGAATATTAATCAGGTTGACAAC  
CGGCAATCTACTAATTTAAAAGATGACATAAACTCAA ACTCTGAGAC  
GGTTGTGACACCCTCAGATATGCCGGATACCAAGCAATTAGTATCAG  
ATGAAACTGACACTCAAAAAGGAGTGACAGAGCCGGATAAGGCGAC  
AAGCCTGCTTGAAGAAAATAAAGGTCCTGTTTCAGATAAAAATACCT  
TAGATTTAAAAGTGGCACCATCTACATTGCAAAATACTCCCGACAAA  
ACTTCTCAAGCTATAGGTGCTCCAAGTCCGACCTTGAAAGTTGCTAAT  
CAAGCTCCACAGATTGAAAATGGTTACTTTAGGTTACATCTTAAAGA  
ATTGCCTCAAGGTCATCCTGTAGAAAGCACTGGGCTTTGGATATGGG  
GAGATGTTGATCAACCGTCTAGTAATTGGCCAAATGGTGCTATCCCT  
ATGACTAATGCTAAGAAAGATGATTACGGTTATTATGTTGATTTTAA  
ATTATCTGAAAAACAACGAAAACAAATATCTTTTTTAATTAATAACA  
AAGCAGGAACAAATTTAAGCGGCGATCATCATATTCCATTATTACGA

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

CCTGAGATGAACCAAGTTTGGATTGATGAAAAGTACGGTATACATAC  
TTATCAGCCCCTCAAAGAAGGGTATGTCCGTATTAACATTTTGAGTTC  
ATCTGGTAACTATGACCACTTATCAGCATGGCTCTTTAAAGATGTTGC  
AACCCCTCAACAACCTTGGCCAGATGGTAGTAATTTTGTGAATCAAG  
GACTATATGGAAGGTATATTGATGTACCACTGAAAATAATGCCAAA  
GAGATTGGTTTTCTAATCTTAGATGAAAGTAAGACAGGAGATGCAGT  
GAAAGTTCAACCAACGACTATGTTTTTAGAGATTTAGCTAACCATTA  
ACCAAATTTTTGTAAAAGATAAGGATCCAAAGGTTTATAATAATCCT  
TATTACATTGATCAAGTGCAGCTAAAGGATGCTCAACAACTGATTT  
AACAAAGTATTCAAGCAAGTTTTACAACCTTAGATGGGGTAGATAAAA  
CTGAAATTTTAAAAGAATTGAAAGTGACAGATAAAAATCAAAATGCT  
ATACAAATTTCTGATATCACTCTCGATACTAGTAAATCTCTTTTAATA  
ATCAAAGGCGACTTTAATCCTAAACAAGGTCATTTCAATATATCTTAT  
AATGGTAACAATGTCACGACAAGGCAATCTTGGGAATTTAAAGACCA  
ACTTTATGCTTATAGTGGAAATTTAGGTGCAGTTCTCAATCAAGATGG  
TTCAAAGGTTGAAGCCAGCCTCTGGTCACCGAGTGCTGATAGTGTC  
CTATGATTATTTATGACAAAGATAATCAAAACAGGGTTGTAGCGACT  
ACCCCTTGTGAAAAATAATAAAGGTGTTTGGCAGACGATACTTGA  
TACTAAATTAGGTATTAATAAATACTGGTTACTATTATCTTTACGA  
AATAAAAAGAGGTAAGGATAAGGTTAAGATTTTAGATCCTTATGCAA  
AGTCATTAGCAGAGTGGGATAGTAATACTGTTAATGACGATATAAAA  
ACGGCTAAAGCAGCTTTTGTAAATCCAAGTCAACTTGGACCTAAAAA  
TTAAGTTTTGCTAAAATTGCTAATTTTAAAGGAAAACAAGATGCTGT  
TATATACGAAGCACATGTAAGAGACTTCACTTCTGATCAATCTTTGG  
ACGGAATAAATAAATAAATAAATAAATAAATAAATAAATAAATAAATA  
AACTAGATTATTTACAGAAATTAGGAGTTACACACATTCAGCTTTT  
ACCGGTATTGAGTTATTTTTATGTTAATGAAATGGATAAGTCACGCTC  
AACAGCTTACACTTCCTCAGACAATAATTACAATTGGGGCTATGACC  
CACAGAGCTATTTTGCTCTTTCTGGAATGTATTCAGAGAAACCAAAA  
GATCCATCAGCACGTATCGCCGAATTAACAATAATACATGATAT  
TCATAAACGTGGCATGGGGGTTATACTTGATGTCGTCTATAATCACA  
CTGCAAAAACCTTATCTCTTTGAGGATATAGAACCTAATTATTACT  
TTATGAATGAAGATGGTTCACCAAGAGAAAGTTTTGGAGGGGGACGT  
TTAGGAACCACTCATGCAATGAGTCGTCGTGTTTTGGTTGATTCCATT  
AAATATCTTACAAGTGAATTTAAAGTTGATGGTTTCCGTTTTGATATG  
ATGGGAGATCATGATGCGGCTGCGATTGAATTAGCTTATAAAGAAGC  
TAAAGCTATTAATCCTAATATGATTATGATTGGTGAGGGCTGGAGAA  
CATTCCAAGGCGATCAAGGTAAGCCGGTTAAACCAGCTGACCAAGAT  
TGGATGAAGTCAACCGATACAGTTGGCGTCTTTTCAGATGATATTCGT  
AATAGCTTGAAATCTGGTTTTCCAAATGAAGGTACTCCAGCTTTCATC  
ACAGGTGGCCCAACAATCTTTACAAGGTATTTTAAAAATATCAAAGC  
ACAACCTGGGAATTTTGAAGCAGATTTCGCCAGGAGATGTGGTGCACT  
ATATTGCTGCACATGATAACCTTACCTTGCATGATGTGATTGCAAAAT  
CAATTAATAAAGACCCTAAGGTAGCTGAAGAAGATATTCATAGACGT

FIG. 1CONT'D

CTGCGTTTAGGAAATGTAATGATTTTAAACATCTCAAGGGACAGCATT  
CATTCAATTCTGGTCAAGAGTATGGTCGTACGAAGCGTTTACTTAACCC  
TGATTACATGACAAAAGTTTCAGATGACAAATTGCCTAATAAAGCAA  
CACTTATTGAAGCTGTTAAAGAATACCCATATTTTATTCATGATTCAT  
ATGATTCTTCAGATGCCATTAATCATTTTGATTGGGCAGCAGCCACAG  
ATAATAACAAACACCCAATTTCAACGAAAACACAGGCCTATACAGCA  
GGTTTAATCACATTAAGGCGTTCAACAGATGCTTTCCGGAAATTGAG  
CAAAGCAGAAATTGATCGTGAGGTTAGCTTGATTACAGAGGTAGGTC  
AAGGTGATATTAAAGAAAAAGATTTGGTTATTGCTTACCAAACAATA  
GATTCTAAAGGCGATATTTACGCAGTATTTGTAAATGCTGATAGTAA  
AGCTAGAAACGTTTTACTAGGTGAAAAATATAAACACCTTTTAAAAG  
GGCAAGTAATTGTTGATGCTGATCAAGCGGGGATTAAACCAATCTCA  
ACTCCTAGAGGTGTTTCATTTTGAAAAAGATAGTTTGCTGATTGATCCA  
TTAACAGCAATTGTGATTAAAGTTGGCAAAGTTGCTCCTAGCCCTAA  
GGAGGAATTGCAAGCAGATTATCCCAAACACAATCTTTCAAGGGAT  
CTAAAACGGTAGAAAAAGTAAATAGAATAGCTAATAAGACCTCAAT  
AACTCCTGTAGTTTCTAATAAGACCGATTTCATATCTGACAAATGAAG  
CTAATTTGCCAAAACTGGAGATAAGTCATCAAAAATACTAAGTGTA  
GTAGGAATAAGCATTCTAGCAAGTCTACTTGCTCTACTAGGTCTCTCT  
TTAAAGAGGAATCGCACTTAA

MKRKDLFGDKQTQYTIRKLSVGVASVATGVCIFLHSPQVFAEEVSVSPA  
TTAIAKSNINQVDNRQSTNLKDDINSNSETVVTSPDMPDTKQLVSDETDT  
QKGVTEPDKATSLLEENKGPVSDKNTLDLKVAPSTLQNTDPDKTSQAIGA  
PSPTLKVANQAPQIENGYFRLHLKELPQGHVESTGLWIWGDVDQSSN  
WPNGAIPMTNAKKDDYGYVDFKLSEKQRKQISFLINNKAAGTNLSGDH  
HIPLLRPEMNQVWIDEKYGIHTYQPLKEGYVRINYLSSSGNYDHLAWL  
FKDVATPSTTWPDGSNFVNQGLYGRYIDVPLKTNAKEIGFLILDESKTGD  
AVKVQPN DYVFRDLANHNQIFVKDKDPKVYNNPYYIDQVQLKDAQQT  
DLTSIQASFTTLDGVDKTEILKELKVTDKNQNAIQISDITLDTSKSLLIK  
DFNPKQGHFNISYNGNNVTTRQSWFKDQLYAYSGNLGAVLNQDGSKV  
EASLWSPSADSVTMIYDKDNQNRVVATTPLVKNNKGVWQTILDTKLGI  
KNYTGYYLYEIKRGKDKVKILDPYAKSLAEWDSNTVNDDIKTAKAAF  
VNPSQLGPKNLSFAKIANFKGKQDAVIYEAHV RDFTSDQSLDGKLKNQL  
GTFAAFSEKLDYLQKLGVTHIQLLPVLSYFYVNEMDKSRSTAYTSSDNN  
YNWGYDPQSYFALSGMYSEKPKDPSARIAELKQLIHDHKGGMGVILDV  
VYNHTAKTYLFEDIEPNYYHFMNEDGSPRESFGGRLGTTTHAMSRRVL  
VDSIKYLTSEFKVDGFRFDMMGDHDAAAIELAYKEAKAINPNMIMIGEG  
WRTFQGDQGKPKPADQDWMKSTDTVGVFSDDIRNSLKSFGPNEGTPA  
FITGGPQSLQGIFKNIKAQPGNFADSPGDVVQYIAAHDNLTLDHVIAXSI  
NKDPKVAEEDIHRRRLRLGNVMILTSQGTAFIHSGQEYGRTRLLNPDYM  
TKVSDDKLPNKATLIEAVKEYPYFIHDSYDSSDAINHFDWAAATDNKHX  
PISTKTQAYTAGLITLRRSTDAFRKLSKAEIDREVSLITEVGQGDIKEKDL

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

\_\_\_\_\_  
VIAYQTIDSKGDIYAVFVNADSKARNVLLGEKYKHLLKGQVIVDADQA  
GIKPISTPRGVHFEKDSLLIDPLTAIVIKVGKVAPSPKEELQADYPKTQSFK  
GSKTVEKVNRIANKTSITPVVSNKTD SYLTNEANLPKTGDKSSKILSVVG  
ISILASLLALLGLSLKRNRT\*

ID-6

Clone 9

ATGAAAAAAGTTTTTTTTCTCATGGCTATGGTTGTGAGTTTAGTAATGATAGCAGG  
GTGTGATAAGTCAGCAAACCCCAAACAGCCTACGCAAGGCATGTCAGTTGTAACC  
AGCTTTTACCCAATGTATGCGATGACAAAAGAAGTATCTGGAGACCTAAATGATGT  
GAGGATGATCCAATCAGGTGCAGGCATTTCATTCCTTTGAACCGTCTGTAAATGATG  
TGGCAGCTATTTATGACGCGGATTTGTTTGTTTACCAATCACATACCTTAGAAGCTT  
GGGCAAGGGATCTAGACCCTAATTTAAAAAAATCAAAGGTTAATGTGTTTGAAGC  
GTCAAAACCTCTGACACTAGATAGAGTCAAAGGGCTAGAAGATATGGAAGTCACA  
CAAGGCATTGACCCTGCGACACTTTATGACCCACATACCTGGACGGATCCCGTTTT  
AGCTGGTGAGGAAGCTGTTAATATCGCTAAAGAGCTAGGACATTTGGATCCTAAAC  
ACAAAGACAGTTACACTAAAAAGGCTAAGGCTTTCAAAAAAGAAGCAGAGCAACT  
AACTGAAGAATACACTCAAAAATTTAAAAAGGTGCGCTCAAAAACATTTGTGACG  
CAACACACGGCATTTTCTTATCTGGCTAAACGATTCGGCTTGAAACAACCTTGGTAT  
CTCGGGTATTTCTCCAGAGCAAGAGCCCTCTCCTCGCCAATTGAAAGAAATTCAAG  
ACTTTGTTAAAGAATACAACGTCAAGACTATTTTGCAGAAGACAACGTCAACCCC  
AAAATTGCTCATGCTATTGCGAAATCAACAGGAGCTAAAGTAAAGACATTAAGTC  
CACTTGAAGCTGCTCCAAGCGGAAACAAGACATATCTAGAAAATCTTAGAGCAAA  
TTTGAAGTGCTCTATCAACAGTTGAAGTAA

MKKVFFLMAMVVSLVMIAGCDKSANPKQPTQGMSVVTSFYPMYAMTKEVSGDLND  
VRMIQSGAGIHSFEPVNDVAIYDADLFVYQSHTLEAWARDLDPNLKSKVNVFEAS  
KPLTLDRVKGLEDM EVTQGIDPATLYDPHTWTD PVLAGEEAVNIAKELGHLDPKHKD  
SYTKKAKAFKKEAEQLTEEYTKQFKKVRSKTFVTQHTAFSYLAKRFGKQLGISGISPE  
QEPSRQLKEIQDFVKEYNVKTIFAEDNVNPKIAHAIAKSTGAKVKTLSPLEAAPSGNK  
TYLENLRANLEVLYQQLK\*

ID-7

Clone 15

TTGTTCAATAAAATAGGTTTTAGAACTTGGAATCAGGAAAGCTTTG  
GCTTTATATGGGAGTGCTAGGATCAACTATTATTTTAGGATCAAGTCC  
TGTATCTGCTATGGATAGTGTTGGAATCAAAGTCAAGGTAATGTTTT  
AGAGCGTCGCCAACGTGATGCGGAAAACAAAAGTCAGGGTAATGTT  
TTAGAGCGTCGCCAACGTGATGCGGAAAACAAGAGCCAAGGCAATG  
TTTAGAGCGTCGTCAACGCGATGTTGAGAATAAGAGCCAAGGCAAT  
\_\_\_\_\_

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

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GTTTTAGAGCGTCGTCAACGTGATGCGGAAAACAAAAGTCAGGGCA  
ATGTTCTAGAGCGCCGCCAACGTGATGCGGATAACAAGAGCCAAGTA  
GGTCAACTTATAGGGAAAAATCCACTTTTTTCAAAGCCAAGTGTATCT  
AGAGAAAATAATCACTCTAGTCAAGGTGACTCTAACAAACAGTCATT  
CTCTAAAAAAGTATCTCAGGTTACTAATGTAGCTAATAGACCGATGT  
TAACTAATAATTCTAGAACAATTTCAAGTGATAAATAAATTACCTAAA  
ACAGGTGGTGATCAAAATGTCATTTTTTAAACTTGTAGGTTTTGGTTTA  
ATTTTGTTAACAAGTCGCTGCGGTTTGAGACGCAATGAAAATTAA

MFNKGFRTWKSGKLWLYMGVLGSTIILGSSPVSAMDSVGNQSQGNVL  
ERRQRDAENKSQGNVLERRQRDAENKSQGNVLERRQRDVENKSQGNV  
LERRQRDAENKSQGNVLERRQRDADNKSQVGLIGKNPLFSKPTVSREN  
NHSSQGDSNKQSFSKKVSQVTNVANRPMLTNNRSRTISVINKLPKTGGDQ  
NVIFKLVGFGILLTSRCGLLRNEN\*

ID-8

Clone 17

ATGACAAAAAACTTATTATTGCTATATTAGCACTATGCACTATCTTAACCACTTCT  
CAAGCTGTTTTAGCTAAAGAAAAATCACAACTGTTACCATAAAAAACAACCTATTC  
GGTCTATATTAAGAAAAAGAAAAAGAGACAAGCCGGATAATAAAAAAGCAAATCAG  
CGAGACACTTAAAGTTCCTTTAAACCCAAAAAAGTAGTTGTTTTTGATATGGGAG  
CTTTGGATACTATCACAGCTTTAGGAGCTGAAAAATCTGTTATTGGTATCCCGAAG  
GCTAAAAATGCTCTAAGTTTATTGCCCAATAACGTCAAATCTGTTTATAAAGCTAA  
GAGATACCAAGACGTAGGAAGTCTCTTCGAACCAAACTTTGAAGCTATTGCTCGTA  
TGCAACCTGATGTGGTTTTCTAGGAGCACGTATGGCTTCTGTTGATAATATTGAA  
AAATTAAGGAGGCTGCACCTAAAGCAGCATTAGTATATGCTGGAGTCGACTCAA  
AAAAAGTATTTGACAAAGGAGTTGCTGAGCGTGTCACAATGTTAGGGAAAAATCTTC  
GACCAAAATAAAAAAGGCAAAAACCTTTAATAAAGATATCGCACAAAGCTGTTCTTA  
AATTGCAGAAAACTATTGAGAAAAAAGGTAAACCTACAGCTCTATTTGTAATGGC  
AAACAGCGGTGAACTTTTAACTCAATCACCTTCTGGTCGTTTTGGTTGGATTTTCTC  
TGTAGGTGGATTTAAGCAGTCAATGAAAATGAAAACTAAGTTCACATGGTACTC  
CCGTATCTTATGAATACATCGCTGAAAAAAATCCTAACTATCTCTTTGTTTTAGATC  
GTGGAGCGACTATTGGACAAGGAGCTTCATCAAAAGAACTTTTTAATAACGATGTT  
ATTAAAGCAACTGATGCTGTCAAAAACAAACGTGTTTCATGAGGTAGATGGAAAAG  
ATTGGTATATCAATTCAGGCGGAAGCCGAGTAACACTCCGTATGATTAAAGATGTA  
CAGAACTTTGTTGATAATCGTTAA

MTKKLIHAILALCTILTTSQAVLAKEKSQTVTIKNNYSVYIKKEKRDKPDN  
KKQISETLVPLPKKVVFDMGALDTITALGAEKSVIGIPKAKNALSLL  
PNNVKSVMYKAKRYQDVGSLEPNFEAIARMQPDVFLGARMASVDNIE  
KLKEAAPKAALVYAGVDSKKVFDKGVAERVMTMLGKIFDQNKKAKTFN  
KDIAQAVLKLQKTIEKKGKPTALFVMANSCELLTQSPSGRFGWIFSVGG

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FIG. 1CONT'D

SUBSTITUTE SHEET (RULE 26)

### Clone 18

GTGAAGAAAACATATGGTTATATCGGCTCAGTTGCTGCTATTTTACTAGCTACTCAT  
ATTGGAAGTTACCAGCTTGGTAAGCATCATATGGGTCTAGCAACAAAGGACAATC  
AGATTGCCTATATTGATGATAGCAAAGGTAAGGTAAGGCCCTAAAACAAACAA  
AACGATGGATCAAATCAGTGCTGAAGAAGGCATCTCTGCTGAACAGATCGTAGTC  
AAAATTACTGACCAAGGTTATGTTACCTCACACGGTGACCATTATCATTTTTACAAT  
GGGAAAGTTCCTTATGATGCGATTATTAGTGAAGAGTTGTTGATGACGGATCCTAA  
TTACCATTTTAAACAATCAGACGTTATCAATGAAATCTTAGACGGTTACGTTATTA  
AAGTCAATGGCAACTATTATGTTTACCTCAAGCCAGGTAGTAAGCGCAAAAACATT  
CGAACCAAACAACAAATTGCTGAGCAAGTAGCCAAAGGAACTAAAGAAGCTAAA  
GAAAAAGGTTTAGCTCAAGTGGCCCATCTCAGTAAAGAAGAAGTTGCGGCAGTCA  
ATGAAGCAAAAAGACAAGGACGCTATACTACAGACGATGGCTATATTTTTAGTCC  
GACAGATATCATTGATGATTTAGGAGATGCTTATTTAGTACCTCATGGTAATCACT  
ATCATTATATTCCTAAAAAAGATTTGTCTCCAAGTGAGCTAGCTGCTGCACAAGCC  
TACTGGAGTCAAAAACAAGGTCGAGGTGCTAGACCGTCTGATTACCGCCCGACAC  
CAGCCCCAGGTCGTAGGAAAGCCCCAATTCCTGATGTGACGCCTAACCTGGACA  
AGGTCATCAGCCAGATAACGGTGGTTATCATCCAGCGCCTCCTAGGCCAAATGATG  
CGTCACAAAACAAACACCAAAGAGATGAGTTTAAAGGAAAAACCTTTAAGGAACT  
TTTAGATCATCTACACCGTCTTGATTTGAAATACCGTCATGTGGAAGAAGATGGGT  
TGATTTTTGAACCGACTCAAGTGATCAAATCAAACGCTTTTGGGTATGTGGTGCCT  
CATGGAGATCATTATCATATTATCCCAAGAAGTCAGTTATCACCTCTTGAAATGGA  
ATTAGCAGATCGATACTTAGCCGGCCAAACTGATGACAACGACTCAGGTTGAGATC  
ACTCAAACCATCAGATAAAGAAGTGACACATACCTTTCTTGGTCATCGCATCAAA  
GCTTACGGAAAAGGCTTAGATGGTAAACCATATGATACGAGTGATGCTTATGTTTT  
TAGTAAAGAATCCATTCATTCAGTGGATAAATCAGGAGTTACAGCTAAACACGGA  
GATCATTTCCTATATAGGATTTGGAGAACTTGAACAATATGAGTTGGATGAGGT  
CGCTAACTGGGTGAAAGCAAAAAGGTCAAGCTGATGAGCTTGTTGCTGCTTTGGATC  
AGGAACAAGGCAAAGAAAAACCACTCTTTGACACTAAAAAAGTGAGTCGCAAAGT  
AACAAAAGATGGTAAAGTGGGCTATATTATGCCAAAAGATGGCAAGGACTATTTT  
TATGCTCGTTATCAACTTGATTTGACTCAGATTGCCTTTGCCGAACAAGAACTAATG  
CTTAAAGATAAGAAGCATTACCGTTATGACATTGTTGATACAGGCATTGAGCCACG  
ACTTGCTGTAGATGTGTCAAGTCTGCCGATGCATGCTGGTAATGCTACTTACGATA  
CTGGAAGTTCGTTTGTATCCACATATTGATCATATCCATGTCGTTCCGTATTTCAT  
GGTTGACGCGCAATCAGATTGCAACAATCAAGTATGTGATGCAACACCCCGAAGT  
TCGTCCGGATGTATGGTCTAAGCCAGGGCATGAAGAGTCAGGTTCCGGTCATTCCAA  
ATGTTACGCCTCTTGATAAACGTGCT

FIG. 1 CONT'D

GGTATGCCAAACTGGCAAATTATCCATTCTGCTGAAGAAGTTCAAAAAGCCCTAGC  
AGAAGGTCGTTTTGCAGCACCAGACGGCTATATTTTCGATCCACGAGATGTTTTGG  
CAAAAGAAACTTTTTGTATGGAAAGATGGCTCCTTTAGCATCCCAAGAGCAGATGGC  
AGTTCATTGAGAACCATTAATAAATCCGATCTATCCCAAGCTGAGTGGCAACAAGC  
TCAAGAGTTATTGGCAAAGAAAAATGCTGGTGATGCTACTGATACGGATAAACCT  
GAAGAAAAGCAACAGGCAGATAAGAGCAATGAAAACCAACAGCCAAGTGAAGCC  
AGTAAAGAAGAAAAAGAATCAGATGACTTTATAGACAGTTTACCAGACTATGGTC  
TAGATAGAGCAACCCTAGAAGATCATATCAATCAATTAGCACAAAAAGCTAATAT  
CGATCCTAAGTATCTCATTTTCCAACCAGAAGGTGTCCAATTTTATAATAAAAAATG  
GTGAATTGGTAACTTATGATATCAAGACACTTCAACAAATAAACCCCTAA

MKKTYGYIGSVAAILLATHIGSYQLGKHHMGLATKDNQIAYIDDSKGKVKAPKTNKT  
MDQISAEEGISAEQIVVKITDQGYVTSHGDHYHFYNGKVPYDAIISELLMTDPNYHFK  
QSDVINEILDGYVIKVNNGNYVYLKPGSKRKNIRTKQQIAEQVAKGTKEAKEKGLAQV  
AHLKKEEVAAVNEAKRQGRYTTDDGYIFSPTDIIDDLGDAYLVPHGNHYHYIPKKDLS  
PSELAAAQAYWSQKQGRGARPSDYRPTAPGRRKAPIPDVTPNPGQGHQPDNGGYHP  
APPRPNDASQNKHQRDEFKKGKTFKELLDHLHRLDLKYRHVEEDGLIFEPTQVIKSNF  
GYVVPHGDHYHIIPRSQLSPEMELADRYLAGQTDDNDSGSDHSPDKEVTHFTFLGH  
RIKAYGKGLDGKPYDTS DAYVFSKESIHSVDKSGVTAKHGDHFHYIGFGELEQYELDE  
VANWVKAKGQADELVAALDQEQGKEKPLFDTKKVSRKVTKDGKVG YIMPDKDGKY  
FYARYQLDLTQIAFAEQELMLKDKKHRYDIVDTGIEPRLAVDVSSLPMHAGNATYD  
TGSSFVIPHIDHIHVVPYSWLTRNQLATIKYVMQHPEVRPDVWSKPGHEESGSVIPNVTP  
LDKRAGMPNWQIIHSAEEVQKALAEGRFAAPDGYIFDPRDVLAKETFWKDGFSFSIPR  
ADGSSLRTINKSDLSQAEWQQAQELLAKKNAGDATDTDKPEEKQQADKSNENQQPSE  
ASKEEKESDDFIDSLPDYGLDRATLEDHINQLAQKANIDPKYLIFQPEGVQFYNNKNGEL  
VTYDIKTLQQINP\*

ID-10

Clone 22

ATGATACGCCAGTTTTTAAGAGAACACTTGATTGTTGTTATATTTTATATATCATGATG  
TTTGTCCTATTTTTTATTAGTTTCTATCTATATCATTTACCAATGCCCTATTTGTTA  
ATTCCTTAGGTTTAAATGTTATTGTTTTACTAGGAATTAGTATTTGGCAATACAGTC  
GTTACAGGAAAAAAATGTTACATCTCAAATATTTTAATAGTAGTCAGGACCCCTCT  
TTCGAACCTTCAACCGAGTGATTACGCTTATTTTAATATTATTACACAATTAGAAGCT  
AGAGAAGCGCAAAAAGTTTCTGAAACAATTGAACAAACCAATCATGTTGCACTTA  
TGATAAAGATGTGGTCGCACCAAATGAAAGTTCCATTGGCAGCTATTTTATTAATG  
GCCAGACAAATCATCTCGATCCTAAGGAAGTTGAACAACAATTATTGAAATTGCA  
ACATTATCTTGAAACGTTGTTAGCATTTTTGAAATTTAGACAATATCGTGACGATTT  
TCGTTTTGAAGCTGTTAGCCTTAGAGAAGTAGTAGTAGAAATTATAAAATCGTATA  
AGGTTATTTGTCTATCCAAAAGCTTATCTATCATAATTGAAGGCGATAATATCTGG  
AAAACAGACAAAAAGTGGTTAACTTTTGCTCTTTTACAGGTGCTAGATAATGCCAT

FIG. 1CONT'D

SUBSTITUTE SHEET (RULE 26)

AAAATATTCTAATCCTGAGTCAAAGATAATAATAAGCATAGGAGAAGAGAGTATT  
AGAATACAAGACTACGGTATCGGCATACTCGAAGAGGATATCCCTAGACTTTTTGA  
AGATGGCTTTACGGGTTACAACGGTTCATGAGCACCAAAAGGCAACAGGCATGGGG  
TTATATATGACAAAAGAAGTCTTATCTAGTCTGAATTTGTCCATTTCCGGTGGATAGC  
AAAATTAATTATGGGACTGCTGTTTCTATACATAAATAA

MIRQFLREHLIWYILYIMMFVLFISFYLYHLPMPYLFNSLGLNVIVLLGISIWQYSRYR  
KKMLHLKYFNSSQDPSFELQPSDYAYFNIITQLEAREAQKVSETIEQTNHVALMIKMW  
SHQMKVPLAAISLMAQTNHLDPKEVEQQLKLQHYLETLLAFLKFRQYRDDFRFEAV  
SLREVVVEIIKSYKVICLSKSLSIIEGDNIWKTDDKWLTFALSQVLDNAIKYSNPESKIIS  
IGEESIRIQDYGIGILEEDIPRLFEDGFTGYNGHEHQKATGMGLYMTKEVLSSLNLSISV  
DSKINYGTAVSIHKZ

ID-11

Clone 23

ATGACTTATCAAAAAACAGTTGTTTTGGCTGGTGATTATTCCTACATTAGACAAATT  
GAAACCACATTAAAATCTCTCTGTGTCTATCATGAGAATCTCTCAATTTTTATTTTT  
AATCAAGATATTCCTCAAGAATGGTTTTTAGCTATGAAAGATAGGGTTGGACAAAC  
TGGAAATCAAATTCAGGATGTAAAGCTCTTCCATGATCACTTATCCCCAAAATGGG  
AAAATAAAAAAGCTTAATCATATTAATTATATGACCTATGCTCGTTATTCATACCTC  
AGTACATCTCAGCTGATACAGTTTTATATCTTGACTCTGACTTAGTTGTTACTACTA  
ATTTAGATAACCTCTTTCAAATTTCACTAGACAATGCATATTTAGCTGCAGTTCCAG  
CTCTTTTTGGGCTTGGATATGGGTTTAATGCTGGAGTAATGGTAATTAACAACCAA  
CGTTGGCGACAAGAAAATATGACTATTAATTAATTGAAAAAATCAAAGGAAA  
TTGAGAATGCCAACGAAGGGGATCAAACAATTCTTAATCGCATGTTTGAAAATCAG  
GTAATTTATTTAGATGATACCTACAATTTTCAAATTGGTTTTGATATGGGAGCTGCT  
ATCGATGGGCATAAATTTATTTTTGACATCCCAATTACCCCACTCCCCAAAATTATT  
CACTACATTTTCGGGAATCAAACCTTGGCAAACATTATCAAATATGAGACTCCGTGA  
GGTATGGTGGCACTATAATTTACTTGAATGGTCAAGTATCATATCTAGTAAAAAAG  
TATTTGGTTTAGACCACCCAATTAACACAAAATTATCGTCTCAATTTCTTATTG  
CTACAATTCTGATTGTATACCATCTATCTCAGAATTAGTCACTGCCCTTCCAGATT  
GTCTATTTACATTGCATGCACCAACAGTTATGTCTGA

MTYQKTVVLAGDYSYIRQIETTLKSLCVYHENLSIFIFNQDIPQEWFLAMKDRVGQTG  
NQIQDVKLFHDHLSPKWENKLNHINYMTYARYFIPQYISADTVLYLDSDLVVTNLD  
NLFQISLDNAYLAAVPALFGLGYGFNAGVMVINNRWRQENMTIKLIEKNQKEIENAN  
EGDQTILNRMFENQVIYLLDDTYNFQIGFDMGAIDGHKFIFDIPITPLPKIIHYISGIKPW  
QTLNMRRLREVWWHYNLLEWSSISSKKVFGLDHPIKTQNYRLNFLIATTSDCIPSISEL  
VTALPDCLFHIACNTSYV\*

ID-12

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

GTGAAGAAACATATTGTTATATCGGCTCAGTTGCTGCTATTTTACTAGCTACTCAT  
ATTGGAAGTTACCAGCTTGGAAGCATCATATGGGTCTAGCAACAAAGGACAATC  
AGATTGCCTATATTGATGATAGCAAAGGTAAGGTAAGCCCTAAAACAAACAA  
AACGATGGATCAAATCAGTGCTGAAGAAGGCATCTCTGCTGAACAGATCGTAGTC  
AAAATTACTGACCAAGGTTATGTTACCTCACACGGTGACCATTATCATTTTTACAAT  
GGGAAAGTTCCTTATGATGCGATTATTAGTGAAGAGTTGTTGATGACGGATCCTAA  
TTACCATTTTAAACAATCAGACGTTATCAATGAAATCTTAGACGGTTACGTTATTA  
AAGTCAATGGCAACTATTATGTTTACCTCAAGCCAGGTAGTAAGCGCAAAAACATT  
CGAACCAAAACAACAATTGCTGAGCAAGTAGCCAAAGGAACTAAAGAAGCTAAA  
GAAAAAGGTTTAGCTCAAGTGGCCCATCTCAGTAAAGAAGAAGTTGCGGCAGTCA  
ATGAAGCAAAAAGACAAGGACGCTATACTACAGACGATGGCTATATTTTLAGTCC  
GACAGATATCATTGATGATTTAGGAGATGCTTATTTAGTACCTCATGGTAATCACT  
ATCATTATATTCCTAAAAAAGATTTGTCTCCAAGTGAGCTAGCTGCTGCACAAGCC  
TACTGGAGTCAAAAACAAGGTCGAGGTGCTAGACCGTCTGATTACCGCCCGACAC  
CAGCCCCAGGTCGTAGGAAAGCCCCACTTCCTGATGTGACGCCTAACCCTGGACAA  
GGTCATCAGCCAGATAACGGTGGTTATCATCCAGCGCCTCCTAGGCCAAATGATGC  
GTCACAAAACAAACACCAAAGAGATGAGTTTAAAGGAAAAACCTTTAAGGAACTT  
TTAGATCAACTACACCGTCTTGATTTGAAATACCGTCATGTGGAAGAAGATGGGTT  
GATTTTGAACCGACTCAAGTGATCAAATCAAACGCTTTTGGGTATGTGGTGCCTC  
ATGGAGATCATTATCATATTATCCCAAGAAGTCAGTTATCACCTCTTGAAATGGAA  
TTAGCAGATCGATACTTAACCCGGCCAAACTGA

MKKTYCYIGSVAAILLATHIGSYQLGKHHMGLATKDNQIAYIDDSKGKVKAPKTNKT  
MDQISAEEGISAEQIVVKITDQGYVTSHGDHYHFYNGKVPYDAIISEELLMTDPNYHFK  
QSDVINEILDGYVIKVNNGNYVYVLKPGSKRKNIRTKQQIAEQVAKGTKEAKEKGLAQV  
AHLSCKEEVAADVNEAKRQGRYTTDDGYIFSPTDIIDDLGDAYLVPHGNHYHYIPKKDLS  
PSELAAQAAYWSQKQGRGARPSDYRPTAPGRRKAPLPDVTNPGQGHQPDNGGYHP  
APPRNDASQNKHQREDFKGTKFKELLDQLHRLDLKYRHVEEDGLIFEPTQVIKSNAF  
GYVVPBGDHYHIIPRSQLSPLEMELADRYLTRPN\*

ID-13

### Clone 28

ATGGTAAATGATATATTAGAAAGAATGTATAAAGAGAATATTCCAAAATCTTACCT  
TACATCCGTCCCATTAGTTATTTCTCAAAAAGGAAGAACAACCTATTCGTTTAGTAT  
GACTGGTGGTCAACAAATAGATGGAGTGAAATTCACACAGATATATGAGGACTAT  
ATGAAATTACTCAGTCAAGGTAAGGATATCGCAGAGTTATATCAAAAATATTCTAA  
AGAAGAGTTGGCAAATCTAGGCATTAATATTTATCAATCCAATGATATAGAAAGG  
ACTGAGGAAAGAACTTTTGATGAAATTATCAGTTGGGTTTCCAACCCTTATGCAAC  
AAGACCAATTCAAGAAAGGCACACTATTCAATTAGAGCCAACAAGATTTTCTACTA

FIG. 1 CONT'D

GAGGATAAGAAAAGAATTGAAGAAGCTGCAGCTCAAGGACTAAGCGAAATCGAC  
CTTATTGATTTAGTTGACCTATATGATATTAATTTAGACAATAACAAGCGTCAATCGC  
CATATTGTGGGGTTATTGACTAATAACACCCCAAGTAACATACTATTTCCAAGAACA  
ATTAAATAAGGAGTTGCTGTCAATGGCTCACGCTTTAGATAACGTACAACAGGCCT  
TTATTAAATTATTAAGTGAAGAGGAGATACGAAAATTTGCTCTTTAA

MVNDILERMYKENIPKSYLTSVPLVISQKGRTTYFSMTGGQQIDGVKFTQIYEDYMK  
LLSQGKDIAELYQKYSKEELANLGINIYQSNDIERTEERTFDEIISWVSNPYATRPQIQRH  
TIQLEPTRFSLEDKKRIEEAAAQGLSEIDLIDLVDLYDINLDNTSVNRHIVGLLTNNTQV  
TYYFQEQNLKELLSMAHALDNVQQAFIKLLSEEEIRKFAL\*

ID-14

Clone 31

ATGAATAAAAGAAGAAAATTATCAAAATTGAATGTAAAAAACAACATTTAGCTT  
ATGGAGCTATCACTTTAGTAGCCCTTTTTTCATGTATTTTGGCTGTAACGGTCATCT  
TTAAAAGTTCACAAGTTACTACTGAATCTTTGTCAAAAGCAGATAAAGTTCGCGTA  
GCCAAAAAATCAAAAATGACTAAGGCGACATCTAAATCAAAAGTAGAAGATGTAA  
AACAGGCTCCAAAACCTTCTCAGGCATCTAATGAAGCCCCAAAATCAAGTTCTCAA  
TCTACAGAAGCTAATTCTCAGCAACAAGTTACTGCGAGTGAAGAGGCGGCTGTAG  
AACAAGCAGTTGTAACAGAAAATACCCCTGCTACCAGTCAGGCACAACAACCTTA  
TGCTGTTACTGAGACAACTTACAAACCTGCTCAACACCAGACAAGTGGCCAAGTAT  
TGAGCAATGGAAATACTGCAGGGGCGGTGCGATCTGCTGCTGCAGCACAAATGGC  
TGCTGCAACAGGAGTCCCTCAGTCTACTTGGGAACATATTATTGCCCGTGAATCAA  
ATGGTAATCCTAATGTTGCTAATGCCTCAGGGAGCTTCAGGACTTTTCCAAACGAT  
GCCAGGTTGGGGTTCAACAGCTACAGTTCAGGATCAAGTTAA

MNKRRKLSKLNKQHLAYGAITLVALFSCILAVTVIFKSSQVTTESLSKADKVRVAK  
KSKMTKATSKSKVEDVKQAPKPSQASNEAPKSSSQSTEANSQQQVTASEEAAVEQAV  
VTENTPATSQAQQTYAVTETTYKPAQHQTSGQVLSNGNTAGAVGSAAAAQMAAATG  
VPQSTWEHILARESNGNPNVANASGASGLFQTMPGWGSTATVQDQVNSAIKAYRAQG  
LSAWGY\*

ID-15

Clone 32

ATGATTGTTGGACACGGAATTGATTTACAAGAGATAGAGGCGATTACTAAAGCAT  
ATGAGCGTAATCAACGTTTTGCAGAACGCGTTTTGACCGAACAAGAATTGCTTCTT  
TTTAAAGGAATTTCCAATCCCAAGCGTCAGATGTCTTTTTTAACAGGGCGATGGGC  
AGCAAAAGAGGCTTATAGCAAAGCACTTGGAACAGGAATTGGGAAAGTTAATTTT  
CATGATATCGAAATTTTATCGGATGATAAAGGAGCGCCTTTGATTACAAAAGAACC

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

GTTTAATGGAAAATCTTTTGTTCATATCTCATAGTGGTAATTATGCACAAGCTAG  
TGTTATTTTGGAGGAAGAAAAATGA

MIVGHGIDLQEIEAITKAYERNQRFAERVLTEQELLLFKGISNPKRQMSFLTGRWAAKE  
AYSKALGTGIGKVNFDIEILSDDKGAPLITKEPFNGKSFVSISHSGNYAQASVILEEEK\*

ID-16

Clone 35

ATGATTTTTGTTCACAGTGGGGACACATGAACAGCAGTTCAACCGTCTTATTAAAGA  
AGTTGATAGATTAAAAGGGACAGGTGCTATTGATCAAGAAGTGTTCAATCAAACG  
GGTTACTCAGACTTCGAACCTCAGAATTGTCAGTGGTCAAAATTTCTCTCATATGAT  
GATATGAACCTTTACATGAAAGAAGCTGAGATTGTTATCACACATGGCGGCCCCAGC  
GACGTTTATGTCAGTTATTTCTTTAGGGAAATTACCAGTTGTTGTTCTTAGGAGAAA  
GCAGTTTGGTGAACATATCAATGATCATCAAATACAATTTTTAAAAAAAATTGCCC  
ACCTGTATCCCTTGGCTTGGATTGAAGATGTAGATGGACTTGCGGAAGCGTTGAAA  
AGGAATATAGCTACAGAAAAATATCAGGGAAATAATGATATGTTTTGTCATAAATT  
AGAAAAAATTATAGGTGAAATATGA

MIFVTVGTHEQQFNRLIKEVDRLKGTGAIDQEVFIQTGYSDFEPQNCQWSKFLSYDDM  
NSYMKEAEIVITHGGPATFMSVISLGKLPVVVPRRKQFGEHINDHQIQLKKIAHLYPL  
AWIEDVDGLAEALKRNIATEKYQGNNDMFCHKLEKIIGEI\*

ID-17

Clone39

TTGGAAGACAAATTATTCAACAAACATTTTATAGGCATTACTATTTTAACTTTATT  
GTTTATATGGTCTATTATTTGTTACCGTTATCATAGCTTTTATTGCGACTAAAGAG  
TTAGGTGTTAGCACTAGCCAAGCAGGATTAGCAACGGGGATTTATATTGTAGGGAC  
TTTGATTGCTCGTCTTATATTTGGTAAGCAATTAGAAGTTCTAGGACGTAAGTTAGT  
TTTACGTGGAGGGGCTATTTTTTACTTACTAACAACCTTTAGCTTATTTTTATATGCC  
AAGTATCGGAGTAATGTATTTAGTTTCGTTTCCTAAATGGTTTTGGTTATGGCGTCGT  
GTCAACAGCAACTAATACTATTGTAACAGCCTATATACCAGCTGATAAAAGAGGTG  
AGGGGATTAACCTTTTACGGTCTATCAACAAGTTTAGCCGCAGCTATTGGTCCTTTTG  
TAGGAACATTTATGCTAGACAACCTTCATATTAACCTTTAAAATGGTTATTGTATTAT  
GTAGTATTTTAATTGCGATTGTAGTGTGGGAGCATTGTTTTCCAGTCAAAAATA  
TTACTTTAAATCCAGAACAGTTAGCTAAATCAAATCATGGACTATTGATAGTTTC  
ATTGAGAAAAAAGCAATTTTTATCACAATTATTGCATTTTTGATGGGTATCTCCTAT  
GCTTCCGTGTTAGGTTTCCAAAAATTATATACAACAGAAATTAATTTGATGACAGT  
AGGAGCTTATTTCTTTATTGTTTATGCACTTGTCACTTTAACCAGACCATCTAT  
GGGAAGATTAATGGACGCTAAGGGAGATAAGTGGGTGCTTTATCCAAGTTATCTGT  
TCTTAACCTTTGGGACTTGCTTTATTAGGGAGTGCTATGGGAAGTGTTACCTACCTTC

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

TATCAGGTGCTTTGATTGGTTTTGGTTATGGCACCTTTATGTCTTGTGGCCAAGCAG  
CATCAATCAAAGGTGTTGAGGAACATCGTTTCAATACAGCCATGTCAACTTACATG  
ATAGGTCTTGATTTAGGGTTAGGTGCTGGACCTTACATTTTGGGACTTGTTAAAGAT  
GGTTTTCTTGGAGCTGGTGTGCAATCCTTTAGAGAATTATTCTGGATAGCAGCGATT  
ATTCCTGTTGTTTGTGGTATTCTATATTTCTTAAAATCATCTAGACAAGTTGAAACT  
AAAACATA  
TAA

MEDKLFNKHFIGITILNFIVYMVYYLFTVIIAFIATKELGVSTSQAGLATGIYIVGTLIARL  
IFGKQLEVLGRKLVLRGGAIFYLLTTLAYFYMP SIGVMYLVRFNLNGFGYGVVSTATNTI  
VTAYIPADKRGEINFYGLSTSLAAIGPFVGT FMLDNLHINFKMVIVLCSILIAIVVLG  
AFVFPVKNITLNPEQLAKSKSWTIDSFIEKKAIFITIIAFLMGISYASVLGFQKLYTTEINL  
MTVGAYFFIVYALVITL TRPSMGR LMDAKGDKWVLYPSYFLTLGLALLGSAMGSVT  
YLLSGALIGFGYGT FMS CGQAASIKGVEEHRFNTAMSTYMIGLDLGLGAGPYILGLVK  
DGFLGAGVQSFRELFWIAAIPVVC GILYFLKSSRQVETKTIZ

ID-18

Clone 47

ATGAATAGTGAACCTAAAAGTCAGTCAAACGAAGTAAAAAATAGCAAGCAATCAG  
AAGTGAAGAAAGATAAAAAAATGACAAAAAAGAACAATTAGCCTATCTCAAAG  
AGCATGAGCAAGAAATCATAGATTATGTAAAATTACATAACAACCAAATTGAGTC  
CGTTCAATTCGATTGGTCAAGTGTAAGTAGAACAAGCGGGAATGGAAGTCCA  
CAAGGGGGTGATTATAATCTTTCACTGAGAGGAAAGTTAATCATCTACAAAATTC  
AAAATTAATAGTTGATTTTTATTAGCTCATAAAAATGATATCCCAAATATCAAAT  
CAATGGGAATGCTAAATAAGCCATATATACATAAAAATGGTATTTGGCACATTTAT  
GAATAG

MILGGCQMNSEPKSQSNEVKNSKQSEVKKDKKMTKKEQLAYLKEHEQEIIDYVKLHN  
NQIESVQFDWSSVKVEQSGNGTPQGGDYNLSLRGKFNHLQNSKLIVDFYLAHKNDIPN  
IKSMGMLNKPYIHKNGIWHIYEZ

ID-19

Clone 102

ATGAAAAAGATTTCGATTATCAAAGTTTATTA AAAATGATTGTTGTTATTTTGT TTTTA  
ATTAGTGTAGCAGCTAGTTTTTATTTTTTCCACGTTGCCCAAGTTCGAGATGATAAA  
TCCTTTATTTCAAATGGTCAACGTAAGCCTGGAACTCTTTATATGCTTATGATAAA  
TCCTTTGATAAGCTATTAAAGCAAAAAATAGAAATGACAAACCAAATATAAAGC  
AAGTTGCTTGGTATGTTCTGTCTGCTAAGAAAACCTCATAAGACAGTTGTTGTCGTTT  
ATGGTTTTGCGAATAGCAAAGAGAATATGAAGGCATATGGTTGGCTGTTTCATAAG  
TTAGGATACAATGTTCTTATGCCTGACAACATTGCACATGGTGAAAGTCATGGGCA

FIG. 1 CONT'D

GTTGATAGGCTATGGCTGGAACGACCGCGAGAACATTATCAAATGGACAGAAATG  
ATAGTGGATAAGAATCCATCAAGCCAAATTACTTTATTTGGTGTTCATGGGTGG  
AGCAACAGTCATGATGGCTAGTGGTGAAAAATTACCTAGTCAGGTTGTTAATAT  
CATTGAAGATTGTGGTTATTCTAGTGTGGGATGAATTAATAATTCAGGCTAAAG  
AGATGTATGGTTTACCAGCCTTCCCCTCTTATATGAAGTTTCAACAATTTCTAAAA  
TCAGAGCAGGTTTTTCGTATGGACAAGCAAGTAGTGTGCAACAATTGAAAAAGAA  
TAATTTACCAGCCCTCTTTATTCATGGTGATAAGGATAATTTTGTTCACAAGTAT  
GGTTTATGACAACATAAAGCTACAGCAGGTAAGAAAGAGCTTTATATTGTAAAA  
GGGGCAAAACATGCGAAATCTTTTGAAACAGAGCCAGAAAAATATGAGAAACGTA  
TCTCTAGTTTTTTGAAAAAATATGAAAAATAA

MKKIRLSKFIKMIVVILFLISVAASFYFFHVAQVRDDKSFISNGQRKPGNSLYAYDKSFD  
KLLKQKIEMTNQNIQVAVWYVPAKKTKHTVTVVHVHGFANSKENMKAYGWL FHKLG  
YNVLMPDNIAHGESHGQLIGYGWNDRENIKWTEMIVDKNPSSQITLFGVSMGGATV  
MMASGEKLPSQVVNIHEDCGYSSVWDELKFQAKEMYGLPAFPLLYEVSTISKIRAGFSY  
GQASSVEQLKKNL PALFIHGDKNFVPTSMVYDNYKATAGKKELYIVKGAKHAKSF  
ETEPEKYEKRISFLKKYEK\*

ID-20

Clone 120

TTGAGGAGTAATATGGTAAAGACAGCAGTTTTAATGGCGACATACAATGGCGAAA  
AATTTATATCTGAACAACCTTGATTCAATTCGCCAACAGACATTAACCAGATTAT  
GTATTATTGAGGGATGATTGTTCAACGGATGAAACAGTCAATGTCGTCAATAACTA  
TATCGCAAAACATGAGTTAGAAGGCTGGAAAATTGTTAAAAACGACAAAACTTA  
GGCTGGCGTTTTAAATTTTCGTCAATTACTTATTGATGTGTTAGCCTATGAGGTTGAC  
TATGTCTTTTTTAGTGATCAAGATGATATTTGGTATCTTGATAAAAACGAACGACA  
GTTTGCCATTATGTCAGATAACCCTCAAATTGAGGTTTTGAGTGCAGACGTTGATA  
TCAAAACGATGTCTACAGAAGCCAGTGTTCCACATTTTCTAACTTTTCTTCTAGTG  
ATAGAATCAGTCAGTATCCTAAAGTATATGATTATCAAACATTCCGTCCTCGGATGG  
ACCATTGCTATGAAGAGAGATTTTGCGCAAGCTATCGCTTGA

MRSNMVKTAVLMATYNGEKFISEQLDSIRQQTLKPDYVLLRDDCSTDETVNVVNNYI  
AKHELEGWKIVKNDKNL GWRLNFRQLLDVLAYEVDYVFFSDQDDIWYLDKNERQF  
AIMSDNPQIEVLSADVDIKTMSTEASVPHFLTFSSSDRISQYPKVYDYQTFRPGWTIAM  
KRDFAJIAZ

ID-21

Clone 143

ATGATTCATGAGATTCACGATTGTCAATTTATTGAAAAAGGAAGTTACGTTTATTT  
GAATTATATTAATGCTGAGGGCGAGAGAGTAGTTATTATAATCATAGATTTTGTCC

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

GTAGTGTTAGTCCTATTTTATATCGTCTATTTATGATTTTACTTGCACAAGAAGTAC  
CTCACTTGCATGATTACATCTATAATGCAAGAGATGATCACTACGATACTTGGAAG  
TTTAAAGAATTAAAGGAGTCAAACCATCCAGTCCTTTTGGCATTCTCTGAAAGGTG  
GCACGATAGTCGCTTGACTTCTAAAAGCCTTGCAGAATGTTTACAATTAACCGACC  
TTGATGAAGAAGTGAAATCGACCATCATTCAATTAAGACAGTTCGAAAAATCAGTC  
AGAAATCCTTTGGCTCACCTGATTAAACCTTTTGATGAGCAAGAACTATATCGTAC  
AACTCAATTTTCTTCTCAAGCATTTTTAGACCAGATTATCTTCTTGGCAAAGGTAAT  
TGGTGTGAGTATGATACTGTTAATTTTCACTACGATACGGTTAACAAGCTTATTAT  
AAAGATACTTGAGTAA

MIHEIHDCQFIEKGSYVYLYNYINAEGERVVIIIIDFVRSVSPILYRLFMILLAQEVPHLHD  
YIYNARDDHYDTWKFKELKESNHPVLLAFSERWHD SRLTSKSLAECLQLTDLDEEVKS  
TIIQLRQFEKSVRNPLAHLIKPFDEQELYRTTQFSSQAFLDQIIFLAKVIGVEYDTVNFHY  
DTVNKLIKILE\*

ID-22

Clone 1

ATGGTAAAAGTTTCAAATTTAGGGTATCCACGTCTTGGTGAACAGCGCGAATGGAA  
GCAAGCGATCGAAGCTTTCTGGGCAGGGAATCTTGAACAAAAAGATTTAGAAAAA  
CAACTAAAACAATTACGTATCAATCATTAAAGAAACAAAAAGAGGCAGGTATTG  
ACCTTATTCCAGTGGGGGATTTTCTTGTTATGATCATGTTTTGGATTTGTCATTTC  
ATTCAATGTAATCCCAAAGCGTTTCGATGAGTATGAGAGGAATTTAGACCTTTATT  
TTGCTATTGCAAGAGGTGACAAAGATAATGTCGCATCATCTATGAAAAAGTGGTTT  
AATACCAACTACCACTACATAGTCCCAGAATGGGAGGTTGAGACTAAACCTCACTT  
GCAGAATAATTACTTACTTGATCTTTATCTAGAAGCTAGGGAAGTAGTTGGTGATA  
AAGCAAAGCCGGTTATC

MEEIMVKVSNLGYPRLGQREWKQAIEAFWAGNLEQKDLEKQLKQLRINHLKKQKE  
AGIDLIPVGDFSCYDHVLDLSFQFNVIPKRFDEYERNLDLYFAIARGDKDNVASSMKK  
WFNTNYHYIVPEWEVETKPHLQNNYLLDLYLEAREVVGDKAKPVI

ID-23

Clone 2

ATGGTGTTACTTTTATTGCTAATGGTAGCCAAGTCAAGTTTGATGGTTACATGGCTG  
TTTATAACGATACTGACAAAAATAAAATGTTACCAGATATGGAGGAAGGAGAAAG  
TTATCAAGTTAA

MVLLLLLMVAKSSLMVTWLFITILTKIKCYQIWRKEKVIKL

ID-24

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

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Clone 14

ATGAACAAAAAATTTCCGGGATCGGCTTGGCTTCGATTGCAGTACT  
TAGTTTAGCTGCATGTGGACATCGTGGTGCTTCTAAATCTGGTGGTAA  
ATCAGATAGCTTGAAGGTTGCAATGGTAACAGATACCGGTGGTGTG  
ATGATAAATCATTTAACCAATCTGGTTGGGAAGGTATGCAAGCTTGG  
GGCAAGAAGAATGGCCTTAAAAAAGGAGCTGGTTTTGACTATTTCCA  
ATCGGCAAGTGAATCTGATTATGCAACTAACTTAGATACAGCTGTGT  
CTAGTGGTTATAAATTGATTTTCGGTATTGGATTTTCTCTTCATGATG  
CTATTGATAAAGCAGCAGACAATAACAAAGATGTTAATTACGTCATC  
GTTGATGATGTTATTAAGGGAAAGATAATGTTGCAAGTGTTGTCTTT  
GCGGATAATGAATCAGCTTACTTAGCAGGTATTGCAGCCGCTAAAAC  
TACCAAAACAAAAACAGTTGGCTTTGTAGGTGGTATGGAATCTGAGG  
TTATTACCCGTTTTGAAAAAGGTTTTGAAGCAGGTGTCAAATCAGTTG  
ATAAATCAATTAATAAATTAAAGTTGACTATGCTGGTTCATTCGGTGAT  
GCTGCTAAGGGTAAGACAATTGCAGCCGCACAATATGCTTCTGGCGC  
AGATATT

MNKKISGIGLASIAVLSLAACGHRGASKSGGKSDSLKVAMVTDGVD  
DKSFNQSGWEGMQAWGKKNGLKKGAGFDYFQSASESDYATNLDTAVS  
SGYKLIFGIGFSLHDAIDKAADNNKDVNYVIVDDVIKGDNVASVVFAD  
NESAYLAGIAAAKTTKTKTVGFVGGMESEVITRFEKGFEAGVKSVDKSI  
KIKVDYAGSFGDAAKGKTIAAAQYASGADI

## ID-25

## Clone 20

ATGTTACATTCTAAAAAAATACATTCCTTATCGCTTATTGCCGTTCTC  
TCTTTAGCAACATATACGAGTTTACAACCAAATCATGTAGCGGCTGA  
ACAATCACAAAAACATCAACTGTTCTTATGAGTCAAAAAACTATTG  
AACATAAGTTAAAAGTTGCAGATAAAGAAGCTGCTCCTCTCTACGCT  
AAAATCGACCATATCCAACGACATATTGAAGTCAAAAAAGCAAAAG  
ATTTAAAAGTTATTGAATTGTATATTAACAAAGATATCAACCAACTA  
GAGAAGCAAAATAAACGTCTACTAACTAAATTCTATACTTCTATTGA  
TAATCAAACATGGGATAGCACAAAGTGAAGTCAAAAAATTGATTGATA  
AGACAACCCTATCCACTAACGAAAAAGATAGATTAAAATTATATTTT  
GAACAACGTGCTTACCTTGAGACAAGGTTGAACGACCGCTATCAAAA  
ATTTGATAACTCTATTGAAAACCAAAATAAAGAACTAAAAATATTAA  
CGTCAAAAAATAGAAAAAATCTATCAAAAACATGGTATTACAAAAGA  
GGTATTAAAACTTACTATGCTAAAAAACAGTACGAGCTGACTGA

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FIG. 1 CONT'D.

MLHSKKIHSLSLIAVLSLATYTSLOPNHVA AEQSQKTSTVLSQKTIEHK  
LKVADKEAAPLYAKIDHIQRHIEVKKAKDLKVIELYINKDINQLEKQNK  
RLLTKFYTSIDNQTWDSTSEVKKLIDKTTLSTNEKDRLKLYFEQRAYLET  
RLNDRYQKFDNSIENQNKELKILTSKIEKIYQKHGITKEVLKTYYAKKTV  
RAD\*

ID-26

Clone 25

Clone 25 (partial sequence)

CTGAATTCCCAAAAACGCTACAATCAAACCTTGGTATCCTACTTATGGTTTTTCTGAT  
ACTTATGCATTCATGGTTACTAAAGAGTTTGCCAGACAGAATAAAATCACCAAGAT  
CTCTGATCTCAAAAAGTTATCAACAACCTATGAAGGCAGGGGTTGATAGTTCATGGA  
TGAATCGCGAGGGAGATGGATACTGATTTGCTAAAACATACGGTTTTGAATTT  
TCACATATTTACCCTATGCAAATTGGCTTAGTCTATGATGCGGTTGAAAGTAACAA  
AATGCAATCTGTATTAGGCTACTCCACTGACGGTCGTATTTGAGCTATGATTTAG  
AAATTTTAAGGGATGATAAAAAATTCTTTCCTCCTTATGAAGCCTCTATGGTTGTCA  
ACAATTCTATCATCAAAAAAGATCCTAAACTAAAAAAATTACTCCATCGACTCGAT  
GGTAAATCAATTTAAAAACGATGCAAAACCTTAATTATATGGTAGATGATAAACT  
TTTAGAAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCG  
CTCACAATTCCACACAACATACGAGCCGGAAGCATAA

LNSQKRYNQTYPTYGFSPTYAFMVTKEFARQNKITKISDLKKLSTTMKAGVDSSWM  
NREGDGYTDFAKTYGFEFSHIYPMQIGLVYDAVESNKMQSVLGYSTDGRISYDLEILR  
DDKKFFPPYEASMVVNNSIIKKDPKLKKLLHRLDGKINLKTMQNLNYMVDDKLLEAW  
RNHGHSCFLCEIVIRSQFHTTYEPEA\*

ID-29

Clone 37

ATGAAAAAATTACTTTCCTAACATGTCTAATCATGATGTCTTTATGT  
TTAGTGGCATGTACTAAGCAAGCAATGTCGTCTAAGCAAGCAATGTC  
GTCTAAGCAAATTAAGATAAGAATAGTAAAGAAAAGGTGATTACT  
GTTGCAACTTACAGCAAACCTACATCTACCTTTTTAGATTTGATTA  
GATAATGTAAAAGAAAAAGGATATACTTTAAAGGTTGTCATGGTCTC  
TGACTATATTCAGGCTAACATTGCTTTAGAAAACAAAGAACATGATG  
CTAACCTTTTACAACATGAATTTTTCATGAGTATCTTTAATAAGGAAA

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

ATGATGGTCATCTAGTGTCAATTACACCAATTTATCATTTCATTGGCTG  
GTTTTTATGGTCAACATTTGAAAAATATTGCCGAGCTTAAAGACGGT  
GCTAAGGTAGCGATTCCGTCTGATCCTGCCAATATGACTAGAGCTCT  
GCTATTATTGCAAGAAAAGAACTTATCACCTTAAAGAATACGTCCA  
AAAAGACCAAGGCTATCGAAGATATTATTACTAACCCTAAAAAATTA  
CGAATTGAACCTGTAGCATTACTTAACCTCAATCAGGCCTATTTTGAA  
TATGACCTTGTCTTTAATTTCCCTGGATATGTGACAAAAATCAATCTA  
GTTCTTAAAAGGGATAGATTATTATATGAGAAAAAACCAGATATCCG  
TTTTGCAGGTGCCTTGGTAGCTCGTGAAGATAATAAAAAATAGTGATA  
AAATAAAAGTACTTAAAGAAGTACTAACAAGTAAAGAGATTTCGTCA  
CTATATCACTAAGGAGATTCCAAGTGAAGCAGACGTTGCGTTCTAG

MKKLLSLTCLIMMSLCLVACTKQAMSSKQAMSSKQIKDKNSKEKVITV  
ATYSKPTSTFLDLIKDNVKEKGYTLKVVMVSDYIQANIALENKEHDANL  
LQHEFFMSIFNKENDGHLVSITPIYHSLAGFYGOHLKNIAELKDGAQVAI  
PSDPANMTRALLLLQEKKLITLKNSTSKTKAIEDIITNPKKLRIEPVALLN  
LNQAYFEYDLVFNFPGYVTKNLVPKRDRLLYEKKPDIRFAGALVARED  
NKNSDKIKVLKEVLTSKEIRHYITKEIPSEADVAF\*

ID-30

Clone 38

CTGTTGGCTAAGGAAACCACTATGTCTGTCCTTTGGTATCAAAATTCTGCAGAAGC  
CAAGGCTTTATATTTACAAGGTTATAATGTTGCTAAAATGAAGTTAGATGATTGGT  
TACAAAAGCCCAGTGAAAAACCATATTCAATTATCTTAGATTTAGATGAAACAGTT  
TTAGATAATAGCCCATATCAAGCAAAGAATATTAAGATGGCTCTAGTTTCACGCC  
AGAGAGTTGGGATAAATGGGTGCAAAAGAAATCAGCTAAGGCTGTTGCGGGTGCC  
AAAGAATTTTTGAAGTATGCTAATGAAAAGGGAATAAAAAATTTATTATGTCTCAGA  
TCGTACAGATGCTCAAGTTGATGCGACTAAAGAAAATTTAGAGAAGGAAGGTATA  
CCTGTTCAAGGGAAAGACCACTTGCTTTTCTTAAAAAAGGAATGAAATCTAAAGA  
GAGTCGCCGTCAGGCAGTTCAAAAAGATACCAATTTAATTATGCTTTTTGGAGATA  
ATTTAGTTGATTTTGCTGATTTTCTAAATCATCTAGTACAGATAGAGAACAACACTAC  
TAACTAAACTTCAAAGTGAGTTTGGTAGTAAATTTATTGTTTTCCCAAATCCTATGT  
ACGGTTCTTGGGAAAGTGCTATTTATCAAGGAAAACATCTGGATGTTCAAAAACAA  
TTGAAAGAACGACAAAAAATGTTGCATTTCGTATGATTAA

MAKLTVKDVDLKGKKVLVRVDFNVPLKDGVTNDNRITAALPTIKYHIEQGGRILFSH  
LGRVKEEADKEGKSLAPVAADLAAGLQDVVFPVTRGAKLEEAINALEDGQVLLVE  
NTRFEDVDGKKESKNDDELGKYWASLGDGIFVNDAFGTAHRAHASNVGISSNVEKAV  
AGFLLENEIAYIQEAVETPERPFVAILGGSKVSDKIGVIENLLEKADKVLIGGGMTYTFY  
KAQGIEIGTYLEKEDKLDVAKDSZ

ID-31

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

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Clone 41

ATGGATAATAAAGGTAATAACGCCAATGTGATTGATGCAATCGCTGAGGGTGCAA  
GCACAGGTGCACAAATGGCTTTCTCAATTGGTGCTAGTTTGATTGCCTTTGTTGGTT  
TAGTTTCTTTGATTAA

MDNKGNNANVIDAIAEGASTGAQMAFSIGASLIAFVGLVSLI

ID-32

Clone 42

ATGAAAAAGAAAAACAAATCCTCTAACATTGCTATAATTGCAATCTT  
TTTTGCTATTATGCTTGTCATTCATTTTTGTGCATCATTTATTTTAGTT  
TTTGGTTAGTCCCTATTAAACCTACTTTGATGCATATCCCAGTTATTA  
TTGCATCTATAGCCTATGGACCTCGTATTGGTGCAACTCTAGGCGCCT  
TAATGGGGGGGATCAGCGTAGCTAACAGCAGCATTGTTCTATTACCA  
ACGAGTTACCTCTTCTCACCTTTTGTTGAAAATGGTAATTTTTATTCTG  
CTAATTATTGCACTTGTACCACGTATTCTAATCGGGATTATTCCTTAT  
TTCGTTTACAAATTACTACACAACCGCTTTGGTTTGGCTATCTCAGGT  
GCTATAGGCTCTCTAACAAACACAGTATTTGTTTTATCTGGAATTTTT  
ATCTTTTTTTCAAGTACTTATAATGGGAATATCAAGCTAATGCTCGCT  
GGGATTATTTCACTAATTCATTAGCTGAGATGGTCATTGCAGCTATC  
ATTGTATATCTAACTGATCCTCGTATTCTCAATATTAAACATTAA

MKKKNKSSNIAIIAIIFFAIMLVHFLSSSIFSWLVPIKPTLMHIPVILASIA  
YGPRIATLGALMGGISVANSSIVLLPTSYLSPFVENGNFYSLIILVPRILI  
GIIPYFVYKLLHNRFLAISGAIGSLTNTVFVLSGIFIFSSTYNGNIKLML  
AGIISSNSLAEMVIAAIIVYLTDPRI LNIKH\*

ID-33

Clone 43

TTGAATATGACATTACAAGACGAAATCAAAAAACGCCGTACTTTTGCCATCATCTC  
TCACCCGGATGCTGGTAAGACGACTATTACTGAGCAATTATTATTTTGGTGGTG  
AAATTAGAGAAGCAGGGACAGTAAAAGGGAAAAAATCAGGTACTTTTGCAAAGTC  
CGACTGGATGGATATTGAAAAGCAACGGGGTATCTCTGTTACTTCATCTGTTATGC  
AATTTGATTACGCGGGTAAACGTGTAA

MNMTLQDEIKKRRTFAIISHPDAGKTTITEQLLYFGGEIREAGTVKGKKSGETFAKSDW  
MDIEKQRGISVTSSVMQFDYAG

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FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

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KRV

ID-34

Clone 44

ATGGCAGATAAAAAACAGAACATTTAAACTTGTAGGTGCAGGATCTTC  
TAGCACACAAGAAAAAATTGAAAAGCCTGCTCTTTCGTTTATGCAAG  
ATGCGTGGCGTCGCTTGAAAAAAACAAATTAGCAGTAGTTTCACTC  
TATTTATTAGCTCTTTTACTTACTTTTTTCGTTAGCCTCAAATTTATTTG  
TAACTCAGAAGGATGCTAATGGGTTTGATTGAAAAAAGTAACGACA  
TATCGCAACTTACCACCTAAATTGAGTTCAAACCTTCCTTTTTGGAAT  
GGTAGCATTAATCCATCA

MADKNRTFKLVGAGSSSTQEKIEKPALSFMQDAWRRLKKNKLAVVSLY  
LLALLTFSLASNLFVTQKDANGFDSKKVTTYRNLPKLSNLFPWNGSI  
NPS

ID-35

Clone 46

ATGAAAAGAAAACAGTTTATAAAATTAGGAATTGCAACCTTACTAACGGTTATTTT  
GCTTTACACACCAATAAACCTAGCTACAAATCATACCACAGAAAATATTGTTACTG  
CTCAAGAGTATAAAACAAAGAGAATGGTACTTTACCTTTTAA

MKRKQFIKLGATLLTVISLYTPINLATNHTTENIVTAQEYKTKENILFLL

ID-36

Clone 50

ATGTTTTATAATCCTTTACTTTTTATTGTACTAATTACAATTGCTGTATTTTTCTTAG  
CTAAGAAAAAATGGCAATTACCGACATTACTTTTCATTGGTTTGCTATTTATCTATA  
ACCAAGGGCTGTGGGAACAGTTGATTAAT

MFYNPLLFIVLITIAVFFLAKKKWQLPTFTFIGLLFIYNQGLWEQLIN

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FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

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ID-37

Clone 51/52

GTGGTGCAAATAATGAAAAACATATAAAAAGTATCATACCAATAGT  
TCTTATTGGTATGATACTAGGAGGCTGTCAAATGAATAGTGAACATA  
AAAGTCAGTATAATGAAACAAAAAGTAGCAAGCAATCAGAAGTGAA  
GAAAGATAAAAAAATGACAAAAAAGAACAATTAGCTTATCTCAA  
GAGCATGAACAAGAAATAATTGATTTTGTAAAATCTCAGAATAAAAA  
GATAGAATCTGTACAAATTGATTGGAATGATGTTTCGATGGAGTAAAG  
GGGGAAATGGTACACCTCAAGGAGGAGGAGAGGGGATTTTACTTTTT  
GGGGAGATTAATAATGATTCTGAATCAAGTTGGAGAGTTGATATTGA  
TATAGAAAAAGGACGGCTAGACCTAAAAAATATGTATTTAGGACAA  
CCTATACGAATTGGAGGTAAATTATTTGAGTAA

MVQIMKKHIKSIPIVLIGMILGGCQMNSEHKSQYNETKSSKQSEVKKDK  
KMTKKEQLAYLKEHEQEIIDFVKSQNKKIESVQIDWNDVRWSKGGNGT  
PQGGGEGILLFGEINNDSESSWRVDIDIEKGRDLKKNMYLGQPIRIGGKLF  
E\*

ID-38

Clone 53

ATGGAATTTTTGGCTTATAATGCTTTCACAGCAATCGGTGTTTCTATT  
CCGCACGGTAATCATTTCCTTATTCACTATAAGGATATGTCTCCA  
TTAGAGTTAGAAGCAACAAGGATGGTGGCAGAGCATAGAGGACATC  
ATATTGATGCATTAGGGAAAAAAGATTCTACAGAGAAACCAAAGCA  
TATTTCTCATGAACCTAATAAGGAACCTCACACAGAGGAAGAACC  
ATGCAGTAACACCGAAAGACCAACGTAAAGGCAAACCAAATAGCCA  
GATTGTCTACAGTGCTCAAGAAATTGAAGAGGCAAAAAAAGCTGGT  
AAATACACAACATCTGATGGTTACATTTTTGATGCTAAAGATATTAA  
AAAAGATACAGGTACAGGTTATGTCATTCCACATATGACACATGAGC  
ATTGGGTACCAAAGAAAGATTTATCAGAGTCGGAATTAAGCAGCT  
CAAGAATTTCTTTCAGGAAAATCTGAAGCAAATCAAGACAAACCAA  
AACAGGTAAAACAGCTCAAGAAATCTATGAGGCAATTGAACCAAAA  
GCAATTGTTAAACCTGAAGATTTATTATTTGGAATTGCACAAGCGAC  
AGACTATAAGAATGGTACATTTGTAATTCCTCATAAAGATCATTACC  
ATTATGTGGAATTAAGATGGTTTGTATGAAGAAAAAGATCTTTTAGCT  
GATTCAGATAAGACATATTCTTTAGAAGACTATTTAGCTACGGCTAA  
ATATTACATGATGCACCCAGAAAAACGTCCTAAAGTTGAAGGATGGG  
GTAAAGATGCTGAAATTTATAAGGAAAAGGACTCTAATAAAGCAGA  
TAAACCAAGTCCTGCACCAACTGATAATAAATCAACATCAAATTCTA

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FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

GTGACAAAACTTAAGTGCTGCAGAAGTATTCAAACAAGCAAAACC  
AGAAAAAATTGTACCGCTTGATAAAATTGCTGCTCACATGGCATATG  
CAGTTGGATTTGAAGATGATCAATTGATTGTTCTCATCATGATCATT  
ATCATAATGTTCTTATGGCATGGTTTGACAAGGGTGGTTTATGGAAA  
GCACCAGAAGGCTATACATTACAACAACCTCTTCTCAACAATTAAATA  
CTACATGGAACATCCTAATGAATTACCAAAAAGAAAAGGGTTGGGGA  
CACGACAGTGATCATAACAAAGGCTCAAATAAAGACAATAAAGCCA  
AAAATTATGCTCCAGATGAAGAACCTGAAGATTCAGGGAAAGTA  
CACAACCTATGGTTTTTATGATGTTAATAAAGGTTTCAGACGAAGA  
ACCAGAAAAACAAGAAGATGAATCAGAGCTAGATGAATATGAAC  
GGAATGGCACAAAACGCTAAGAAATATGGTATGGATAGACAATCTTT  
TGAAAAGCAACTCATCCAATTATCAAATAAATATAGTGTAAAGTTT  
GAAAGC

MEFLAYNAFTAIGVSIPHGHNHFHFIHYKDMSPLELEATRMVAEHRGHHI  
DALGKKDSTEKPKHISHEPNKEPHTEEHHAVTPKDQRKGKPN SQIVYS  
AQEIEEAKKAGKYTTSDGYIFDAKDIKDTGTGYVIPHMTHEHWVPKK  
DLSESELKAAQEFLSGKSEANQDKPKTGKTAQEIEAIEPKAIVKPEDLL  
FGIAQATDYKNGTFVIPHKDHYHYVELKWFDEEKDLLADSDKTY SLED  
YLATAKYMMHPEKRPKVEGWGKDAEIEYKEKDSNKADKPSPAPTDNK  
STSNSSDKNLSAAEVFKQAKPEKIVPLDKIAAHMAYAVGFEDDQLIVPH  
HDHYHNPMAWFDKGGGLWKAPEGYTLQQLFSTIKYYMEHPNELPKEK  
GWGHDSHDHNKGSNKDNKAKNYAPDEEPEDSGKVTHNYGFYDVNKG  
DEEPEKQEDESELDEYELGMAQNAKKYGM DRQSFEKQLIQLSNKY SV  
SFES

ID-39 (Same as ID-76)

Clone 56

ATGAGGAAACGTTTTTCCTTGCTAAATTTTATTGTTGTTACTTTTATTT  
TCTTTTTCTTTATTCTTTTTCCGCTTTTTAAGGCCAAAGATTGTCAGGT  
TGTTTATGCAAGTTTTCAAGGAGATCATTGGGACATTTGTAACGCATT  
TGATTTTCCGTATTTACATCGCTTTGATCTCATTAAAGGTAAAGAAAA  
TCAACTTTACTTTATAGGTTGTACAATTGCTAACAGTAAAGCCTACAC  
TGAGGATTGGAGTGATAAAGGCCGAATTTTGTGCTCGTTTTAATAC  
TCAAAACCATACATTGGAAGGATTGCAACAATTGCCTCAAACCTTTAT  
TAAAAAATCATGGATACTATGCCATT CAGGATGAAGGATATTCATTG  
ATTACTTCAGTAGAAGGGGTACTCAAACCTCACTTATCCAGAATTTTCT  
ACTACAGGCGACTGGCAATTAGAACGGCTTTTCGATGAGGAGACAAG  
CGATGTGGTGAAAGTGGATATTAATCAGGATGGTAAGGATGAGTATG  
TGATCATCCAAGGTTTTCATGGAGATCGTTTACGTATCTTCACTGAAG  
ATTTCCGGTCGAGAATTATTCCATTATCCTGAAAAAACCCCATTTGGTC  
ACGCTATTTGGAGTGGTCGTTTACTTAATCAGACTTGTTTCGTATTCG

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

GGTGGCGATCAGAAAAAGCAGAATTAAGGCTTTTTCACTTTGTAGAT  
GGGCACTTGGTTTCAGAATTAGTAGATGCAAAAGCAGCTTCTAGTAA  
TGTCTTAGCTTTTGAAAAAGATGGAAAAGCTTATCTTTTCTCAGCCAA  
TAACGGACGTGGCGAAGTTGCTCTTTATCAATTAGTAAAATAA

MRKRFSLLNFIVVTFIFFFFILFPLFKAKDCQVVYASFQGDHWDICNAFDF  
PYLHRFDLIKGENQLYFIGCTIANSKAYTEDWSDKGRIFVARFNTQNHT  
LEGLQQLPQTLLKNHGYIAIQDEGYSLITSVEGVCLKLTYPEFSTTGDWQ  
LERLFDEETSDVVKVDINQDGKDEYVIIQGFHGDRLRIFTEDFGRELPHY  
PEKTPFGHAIWSGRLLNQTCFVFGWRSEKAELRLFHFVDGHLVSELVDA  
KAASSNVLAFEKDGKAYLFSANNGRGEVALYQLVK\*  
ID-40

Clone 57

ATGAAGCACAAGTTAAAAGCTTTTACGCTTGCTTTACTCTCAATATTC  
TTTGTGTTTGGTGGAAAGGTCAGTGCAGAGACTGTGAATATTGTTTCT  
GATACAGCATACGCTCCATTTCGAATTTAAAGATTCTGATCAAACCTTAT  
AAAGGAATCGATGTTGACATCGTTAACGAAGTCGCTAAGCGTGCTGG  
CTGGAATGTTAACATGACGTATCCAGGTTTTGATGCCGCAGTTAACG  
CTGTTCAATCTGGACAGGCAGATGCGCTAATGGCCGGAACACTACTGTT  
ACTGAAGCACGTAAAAAAGTCTTTAATTTCTCAGATACTTATTACGAT  
ACTTCCGTTATTCTTTATACTAAAAATAATAATAAAGTCACAAACTAC  
AAACAACATAAAAGGAAAAGTAGTCGGTGTAATAAATGGAACAGCTG  
CTCAAAGCTTCTTAGAAGAAAATAAATCTAAATACGGCTATAAAGTT  
AAAACATTTGATACAAGCGACCTAATGAATAACAGCCTTGATTCTGG  
TTCTATTTACGCCGCTATGGACGATCAACCAGTTGTGCAATTTGCGAT  
AAATCAAGGAAAAGCTTACGCCATTAACATGGAAGGCGAAGCAGTT  
GGTAGCTTTGCATTTGCTGTCAAAAAAGGTAGTGACACGATAATCT  
AATTAAAGAATTTAACACAGCTTTTGCACAAATGAAATCAGATGGCA  
CTTATAATGACATCATGGATAAATGGCTTGGAAGAGCGCTACAAAA  
ACAAGCGGCAAAGCAACAGGTAATGCCAATGAAAAAGCAACTCCTG  
TAAAGCCAAGTTATAAAATTGTTTCTGATTCTTCATTTCGCACCATTCG  
AATATCAAAACGGTAAAGGGAAATATACTGGTTTTGATATGGAATTA  
ATCACGAAAATTGCTAAACAGCAAGGTTTTAAACTTGATATCTCAA  
TCCAGGTTTTGATGCCGCTTTAAATGCTGTCCAATCTGGGCAAGCTGA  
CGGTGTTATTGCAGGAGCCACAATCACAGAAGCACGCCAAAAAATCT  
TTGATTTTTCTGATCCTTATTACACATCTAGCGTTATCTTAGCGGTTAA  
AAAAGGAAGCAATGTCAAATCATAACCAAGATTTAAAAGGAAAAACA  
GTTGGTGCTAAAAATGGTACTGCCTCATATACTTGGTTATCAGACCAC  
GCAGATAAGTACAACATCATGTAAAGCATTTGATGAAGCATCTAC  
AATGTATGATAGTATGAACTCAGGTTCAATTGATGCTCTAATGGATG  
ACGAAGCCGTTCTTGCTTACGCTATTAATCAAGGTCGTAAATTTGAA  
ACACCTATCAAAGGTGAAAAATCAGGCGATATCGGATTTGCAGTGAA

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

AAAAGGGGGCAAATCCAGAATTAATTAATAATGTTTAACAACGGTCTTG  
CTTCACTCAAAAAATCGGGTGAGTACGATAAACTTGTTAAAAAATAC  
CTTTCCACAGCCAGCACTTCTTCAAACGATAAAGCTGCTAAACCTGT  
AGATGAATCAACTATTTTAGGGTTAATTTCTAATAACTACAAACAATT  
GCTATCTGGTATTGGAACACTTTAAGTTTAACTCTTATCTCGTTTGC  
GATTGCTATGGTATTGGTATTATCTTTGGTATGATGAGCGTATCACC  
AAGTAATACTCTCCGCACAATTTCAATGATTTTTGTTGATATTGTCCG  
TGGTATTCCACTCATGATTGTGGCCGCTTTTATTTTCTGGGGTATTCTT  
AATTTAATCGAAAGCATCACAGGTCACCAAAGTCCAATTAATGACTT  
CGTTGCTGCTACTATCGCTCTTTCTTTAAATGGTGGTGCGTACATTGC  
TGAAATTGTACGTGGTGGTATTGAAGCTGTTCTTCTGGTCAAATGGA  
AGCAAGTCGCAGCTTAGGTATTTCTTACGGCAAACACTATGCAAAAGG  
TTATCTTACCTCAAGCAGTACGCCTTATGTTACCAAACCTTTATCAACC  
AATTTGTATCTCATTAAAGGATACAACAATTGTATCAGCAATCGGA  
CTTGTGGAACCTCTTCCAAACTGGTAAATCATAA

MKHKLKAFTLALLSIFVFGGKVS AETVNIVSDTAYAPFEFKDSDQTYK  
GIDVDIVNEVAKRAGWNVNMTYPGFDAAVNAVQSGQADALMAGTTV  
TEARKKVFNFSDTYYDTSVILYTKNNNKVTNYKQLKGKVVGKNGTA  
AQSFLEENKSKYGYKVKTFTDSDLMNNSLDSGSIYAAMDDQPVVQFAI  
NQGKAYAINMEGEAVGSFAFAVKKGSGHDNLIKEFNTAFAQMKSDGTY  
NDIMDKWLKDATKTSGKATGNANEKATPVKPSYKIVSDSSFAPFEYQ  
NGKGKYTGFDMEITKIAKQQGFKLDISNPGFDAALNAVQSGQADGVIA  
GATITEARQKIFDFSDPYTSSVILAVKKGSNVKSYQDLKGKTVGAKNG  
TASYTWLSDHADKYNHYHVKAFDEASTMYDSMNSGSIDALMDDEAVLA  
YAINQGRKFETPIKGEKSGDIGFAVKKGANPELIKMFNNGLASLKSGEY  
DKLVKKYLSTASTSSNDKAAKPVDESTILGLISNNYKQLLSGIGTTLSLT  
ISFALAMVIGIIFGMMSVSPSNTLRITISMIFVDIVRGIPLMIVA AFIFWGIPN  
LIESITGHQSPINDFVAATIALSLNGGAYIAEIVRGGIEAVPSGQMEASRSL  
GISYGKTMQKVILPQAVRLMLPNFINQFVISLKDTTIVSAIGLVELFQTGK  
S\*

ID-41

Clone 58

TTGGAAGGTTTACTTATTGCATTGATTCCCATGTTTGCGTGGGGAAGTATTGGATT  
GTTAGTAATAAAATTGGAGGGCGTCCAAATCAACAAACATTTGGAATGACTTTAGG  
AGCATTGCTATTTGCGATTATCGTATGTTTATTAA

MEGLLIALIPMFAWGSIGFVSNKIGGRPNQQTFGMTLGALLFAIIVCLF

**FIG. 1** CONT'D

27 / 60

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ID-42

Clone 70

ATGAATACTATTTATAATACATTGAGAACAGATAAAGGTTATAAAGT  
TTATGAGGGGTATTTATATGAAATTACTGGTGAAGAATGTGAAGAAG  
CCTTAGACCTTGTGATTCCCTAAGAATATTGTATTTGCAGATACAGATA  
CTTGTGGCTACACTTTTTTACTCAATGAAGATGGAACAGTTTATGATG  
ATGTGACTTTCTACAAATTTGATGATAAATATTGGTTGGCTAGTCATA  
AAGCTTTGGATTCTTATTTAGACAACATCAATTTTGACTATACCGTAA  
CAGATATTTCTGACGAGTATAAAATGCTGCAAATTGAAGGAAGATAT  
TCGGGAGAAATTGCTCAGTCATTTTATGAATATGATATTTCAACACTT  
AATTTTCGTA CTCTTCGCATAGAGATGGACTTCATCAAAGGTGAGGA  
AAGGTTATCTTGGCGTAGATTTGGTTTTCTGGAGAATTTGGCTATCA  
ATTTTTCCTACCATCTTCTATTTTGGTACTTTTGTTTCGGATGTCTGT  
GAAGGTATAGCAGAGTGTGGGGATGAACTTGATAGATATTTAAGGTT  
TGAAGTGGGACAACCCATTACTGATATTTATCAACAAGAAGAATATT  
CTTTATATGAAATAGGTTATTCTTGGAAATCTAGATTTCAAAAGGAA  
GAATTTAGAGGTCGCGATAGCTTGTTAGAGCACATCAGATCAGCAAC  
AGTTAAAAGTGTTGGATTCTCAACGAAGGAAAAACTCGCTTCAGGAA  
CACCAGTGCTATTTGATGACCAAATTGTTGGAAAGATTTTTTGGATAG  
CAGACGAGAAACACTCTTCGGAAAATTACCTAGGTTTGATGATTGTT  
AACCAAACATATGCTCATTGAGGAGTTACTTTTGTAACAGAAGATGG  
CCAAATTTTGAAAACACAATCAAGCCCTTATTGTATCCCAGAAAGTT  
GGAACAAAGAATGA

MNTIYNLRTDKGYKVYEGYLYEITGEECEEALDLVIPKNIVFADTDTCG  
YTFLLNEDGTVYDDVTFYKFDDKYWLASHKALDSYLDNINFDTYTVTDIS  
DEYKMLQIEGRYSGEIAQSFYEYDISTLNFRTLRIEMDFIKGEERLSWRRF  
GFSGEFGYQFFLPSSIFATFVSDVCEGIAECGDELDRYLRFVVGQIPITDIY  
QEEYSLYEIGYSWNLDFTKEEFRGRDSLLEHIRSATVKS VGFSTKEKLA  
SGTPVLFDDQIVGKIFWIADEKHSSENYLGLMIVNQTYAHSGVTFVTED  
GQILKTQSSPYCIPESWNKE\*

ID-43

Clone 78/94

ATGGAGTTAGTAATTAGAGATATTCGTAAGCGGTTTCAGGAAACAGA  
GGTCTTGAGAGGAGCAAGTTACCGATTTTATTCAGGTAAAATAACAG  
GGGTCTTAGGTAGGAATGGTGCTGGGAAAACAACCTTTATTTAATATA  
CTTTATGGGGATCTTGCAGCTGACAACGGGACCATTGTTTATTGAAG  
GATAATCACGAGTATCCTCTTACCGATAAGGATATTGGTATTGTTTAT

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FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

TCCGAAAACTACCTTCCAGAATTTTAAACAGGGTATGAATTTGTAAA  
ATTTTACATGGATTTACATCCTTCAGATGATTTAATGACAATAGATGA  
TTATTTAGATTTTATGGAAATAGGACAAACAGAGCGTCATAGAATTA  
TCAAAGGATATTCTGATGGAATGAAGAGTAAGCTCTCATTAAATTTGC  
CTGATGATTTCTAAGCCAAAAGTAATTTTACTAGATGAGCCACTGAC  
TGCAGTTGATGTTGTATCAAGTATTGCAATAAAACGCCTTTTGTGGA  
ATTAAGTGAGGATCATATTATTATATTATCAACTCATATAATGGCCTT  
AGCAGAAGATCTATGTGATATTGTGGCTGTATTAGACAAAGGAAAAC  
TCCAAACATTAGATATTGATCGTAAACATGAACAATTCGAAGAGCGT  
CTTCTTCAAGTGTTGAAGGGAGATGAATATGACAAGTAA

MELVIRDIRKRFQETEVLRGASYRFYSGKITGVLGRNGAGKTTLFNILYG  
DLAADNGTICLLKDNHEYPLTDKDIGIVYSENYLPEFLTGYEFVKFYMD  
LHPSDDLMTIDDYLDMEIGQTERHRIKGYSDGMKSKLSLICLMISKPK  
VILLDEPLTAVDVVSSIAIKRLLLELSEDHIIILSTHIMALAEDLCDIVAVL  
DKGKLQTLDIRKHQFEERLLQVLKGDEYDK\*

ID-44

Clone 80

TTGTTTATGAGATATACAAATGGAAATTTTGAAGCCTTTGCAAGACCT  
CGAAAACCTGAAGGTGTGGATAAAAAATCCGCTTATATTGTTGGTTC  
TGGTTTAGCAGGATTAGCTGCCGCTGTCTTTTAAACGTGACGGTCA  
AATGGATGGTCAACGTATTCATATTTTGAAGAACTACCTCTTTCTGG  
AGGATCACTTGACGGTGTCAAACGACCTGATATCGGTTTTGTAAACGC  
GTGGTGGTCGTGAAATGGAAAATCACTTCGAATGTATGTGGGATATG  
TACCGTTCCATCCCCTCTCTCGAAGTTCAGATGCTTCTTATCTAGAT  
GAATTTTATTGGCTTGACAAGGATGATCCCAATTCATCTAACTGTCGC  
CTCATTCTATAAACAGGGGAATCGCTTAGAATCTGATGGTGATTTTAC  
ACTCGGAACACATTCCAAAGAGTTAGTTAAGCTAGTCATGGAGACTG  
AAGAGTCTTTAGGTGCTAAGACGATTGAAGAAGTTTTTTCAAAGAA  
TTTTTTGAAAGTAATTTTGGACTTATTGGGCTACTATGTTTGCCTTTG  
AGAAATGGCATTGAGCGATTGAAATGCGTCGATATGCTATGCGCTTT  
ATCCATCATATTGGTGGTCTGCCTGATTTCACTTCATTAAAATTTAAT  
AAATATAATCAATATGATTCTATGGTGAAACCAATCATCAGTTATTTA  
GAGTCTCATAATGTAGATGTTCAATTTGATAGCAAGGTAACATAAT  
CTCCGTAGACTTT

MFMYRTNGNFEAFARPRKPEGVDKKSAYIVGSGLAGLAAAVFLIRDGQ  
MDGQRIHIFEELPLSGGSLDGVKRPDIGFVTRGGREMENHFECMWDY  
RSIPSLEVPDASYLDEFYWLKDDPNSSNCRLLHKQGNRLES DGDFTLGT  
HSKELVKLVMETEESLGAKTIEEVFSKEFFESNFWTYWATMFAFEKWHS

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

AIEMRRYAMRFIHHIGGLPDFTSCLKFNKYNQYDSMVKPIISYLESHNVDV  
QFDSKVTNISVDF

ID-45

Clone 81

TTGTTGGCTTCTTTATTTATCGTCCGTTTGTCAAATCGCTTTCGCTAA  
GGAGGAGCAATATGAAAAATTACTTAGATGGCTTCCTCCTGTACTT  
TTCATTATTATCCTTATAGGAATGACTATCTTAGGTAAGTCCTATATC  
AATAAAGTAACAGCTCACAAAATAAACTCTATAACTCTCGAATGAC  
TCCTACTATTTTAATTTTCAGGATCCAGTGCTACTCAAGAACGATTAA  
CAGCATGTTAGCACAGCTCAACCAAATGGGAGAAAAACATAGCGTTT  
TAAAGTTAACTGTCAAAAAAGACAATAGCATTATCTACAATGGACAA  
ATTAGCGGCAATGACCACAAACCCTACATTGTCATTGGATTGAAAA  
TAATGAAGATGGTTATAGTAACATCAAAAAACAAACAAAATGGCTA  
CAGATTGCTATGAATGATCTTCAGAAGAAATATAAATTTAAACGTTT  
TAACGCTATCGGTCATTCAAATGGTGGCTTATCATGGACTATTTTCCT  
AGAAGATTATTACGACTCTGATGAATTTGATATGAAATCATTGTAA  
CAATGGGAACACCTTTTAACTTTGAAGAAAGTAACACCTCAAATCAT  
ACTCAAATGCTTAAAGATTTAATCAGTAATAAAGGAAATATTCCATC  
AAGTCTCATGGTATACAATTTGGCAGGAACTAATTCATATGATGGTG  
ATAAAATTGTTCCATTTGCTAGTGTGGAGACTGGTAAATATATTTTCC  
AAGAAACCGCTAAACACTATACCCAATAACAGTAACTGGTAATAAT  
GCTACACATTCTGACTTGCCTGATAATCCTGAAGTTATCCAATATGTC  
GCAGAAAAAATTCTTAAAAATGAGAAAGGTAAATTACCAAACCTC  
ACTAA

MLASLFIVRLSKSLSLRRSNMKLLRWLPPVLFIIILIGMTILGKSYINKVT  
AHKIKLYNSRMTPTILISGSSATQERFNSMLAQLNQMGKHSVLKLTVK  
KDNSIYNGQISGNDHKPYIVIGFENNEDGYSNIKKQTKWLQIAMNDLOK  
KYKFKRFNAIGHNSNGGLSWTIFLEDYYSDEFDMKSLLTMGTPFNFEES  
NTSNHTQMLKDLISNKGNISSLMVYNLAGTNSYDGDKIVPFASVETGK  
YIFQETAKHYTQLTVTGNNATHSDLPDNPEVIQYVAEKILKNEKGKLPK  
PH

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ID-46

Clone 83

TTGAAATTAGGTATTACAACATTCGGAGAGACAACAATCCTTGAAGAAACAAACC  
AAAGCTATTACATCCTGAGAGGATTCGCCAATTAGTTGCTGAGATTGAACTAGCT  
GATCAAGTTGGTTTAGATGTATATGGTATTGGAGAGCACCATCGTGAAGATTTTGC

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

GGTCTCTGCACCCGAAATTATCCTAGCAGCAGGAGCGGTTAGAACTAATAATATCC  
GTTTATCTAGTGCAGTAACGATTCTCTCTTCCAATGATCCTATTCGCGTCTATCAGC  
AATTTTCAACGATTGACGCACTTTCAAATGGTAGAGCAGAAATTATGGCAGGGCGT  
GGTTCCTTTATTGAGTCTTTTCCATTGTTTGGATACGATTTAGCGGATTATGATGAT  
TTATTTAATGAAAAAATGGATATGTTGTTAGCAATTAAGTCTAGCGACAAATCTCGA  
TTGGAAAGGTCATTTGACACAAACAGTTAATGAGCGACCAATTTATCCAAGAGCAT  
TACAAAGACAGTTATCAATATGGGTGGCAACAGGAGGAAATGTTGATTCTACAATT  
CGTATTGCAGAACAAGGTTTGCCAATTGTTTATGCAACTATTGGTGGGAATCCCAA  
AGCCTTTCGTCAATTGGTCCATATTTATAAAGAAGTTGGTAAGTCCGTAATGGACA  
CAAACCAGGAACAATAAAAGTTGCTGCTCACTCTTGGGGATGGATTGAAGAGGA  
TAATCAAACCGCTATTGACCGTTATTTTTTCCCTACGAAACAGACCGTCGATAATAT  
TGCTAAGGGACGCCCTCATTGGTCTGAAATGACTAAAGAGCAGTATTTACGTTCAA  
TAGGTCCAGAAGGTGCTATTTTTGTAGGAAATCCTGAAGTGGTTGCACATAAAATT  
ATAGGACTTTGGTGA

MKLGITTFGETTILEETNQSYSHPERIRQLVAEIELADQVGLDVYGIGEHHRDFAVSAP  
EILLAGAVRTNNIRLSSAVTILSSNDPIRVYQQFSTIDALSNGRAEIMAGRGSFIESFPLF  
GYDLADYDDLNEKMDMLLAINSATNLWDKGHILTQTVNERPIYPRALQRQLSIWVAT  
GGNVDSTIRIAEQGLPIVYATIGGNPKAFRQLVHIYKEVGKSVMDTNQEQLKVAHSW  
GWIEEDNQTAIDRYFFPTKQTVDNIAKGRPHWSEMTKEQYLRISIGPEGAIFVGNPEVV  
AHKIIGLW

ID-47

Clone 86

ATGATAGAGTGGATTCAAACACATTTACCAAATGTATATCAAATGGG  
TTGGGAAGGTGCTTACGGCTGGCAGACAGCTATTGTACAAACCTTT  
ATATGACTTTTTGGTCGTTCTTATTGGAGGTTTAATGGGATTGTTAG  
GAGGTTTATTCCTTGTTTAACTAGTCCTAGAGGAGTTATTGCTAATA  
AATTAGTATTTGGAGTTTTAGATAAAGTTGTTTCTGTTTTTAGAGCTC  
TGCCCTTCATTATTCTTCTTGCTTTGATTGCGCCAGTAACTCGCGTAAT  
TGTAGGAACAACACTTGGTTCACCAGCAGCTTTGGTACCTCTTTCTTT  
GGCAGTTTTCCCATTTTTTGCTCGTCAAGTTCAAGTTGTTTTAGCTGA  
ACTTGATGGTGGAGTTATTGAGGCTGCACAAGCCTCAGGTGGAACAC  
TTTGGGATATTATTGTAGTTTATCTTCGTGAAGGTCTACCAGATTTAA  
TTCGAGTATCAACGGTTACTTTGATTTCTTTAGTAGGTGAAACAGCTA  
TGGCTGGCGCTATTGGTGCAGGAGGATTGGGTTCTGTTGCTATTACTA  
AAGGATATAACTATTCTCGTGATGATTAATTCTTTAGTAGCGACTATTC  
TGATTTTATTATTAATTTCTTTATCCAATTTTTAGGTGATTTTTTAAC  
ACGTCGCTTGAGTCATAAATAA

MIEWIQTHLPNVYQMGWEGAYGWQTAIVQTLYMTFWSFLIGGLMGLL  
GGLFLVLTSPRGVIANKL VFGVLDKVVSVFRALPFIILLALIAPVTRVIG

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

\_\_\_\_\_  
TTLGSPAALVPLSLAVFPFFARQVQVVLAEVDGGVIEAAQASGGTLWDII  
VVYLREGLPDLIRVSTVTLISLVGETAMAGAIGAGGLGSVAITKGYNYSR  
DDITLVATILILLIFFIQFLGDFLTRRLSHK\*

ID-48 (same as ID-43)

ID-49

Clone 96

TTGGCAGTTAGTTTTTCATGAAGTATTTGGTTGGGATTCTGCTTTTTTTA  
TTATGATTATCAATATTCCATTGCTCCTTCTTTGCTACTTTGGCTTAGG  
TAAACAAACCTTTTTAAAACTGTCTATGGTTCTTGGATTTTTCTGT  
TTTTATTAAGTTAACACAAAGTGTAACCACTTTGACCCACAACTCACT  
CCTCGCAGCACTTTTTGGAGGTGTTATTGTAGGATGTGGTTTGGGGAT  
TGTTTTTTGGAGCGACTCTTCAACTGGTGGAACGGGGATTATCATTCA  
ATTCTTAGGAAAATATACTCCTATAAGCCTTGGACAAGGGGTATAT  
TGATTGATGGACTTGTTACAATTGTTGGTTTCCTAGCTTTTGACAGTG  
ATACGGTTATGTTTTCTATTATTGGGTTGATAACTATTAGTTATATTAT  
TAATGCTATCCAACTGGATTTACAACCTTAAGCACTGTCTTAATCGT  
TTCTCAAGAGCACCAAAAAATTAAGACATATATCAATACTGTGCGCAG  
ATAGAGGAGTAACAGAAATTCCCGTTAAAGGGGGATATTCTGGA  
AATCAAATCATGCTTATGACAACTATTGCTGGTTATGAGTTTGCTAAA  
TTACAAGAGGCAATAGCAGAAATTGACGAAACAGCCTTCATAACAGT  
AACTCCAACATCACAAGCTTCTGGACGTGGATTTAGTCTTCAAAAA  
ATCATGGACGTCTTGATGAAGACATTCTTATGCCAATGTAA

MAVSFHEVFGWDSAFFIMIINPLLLLCYFGLGKQTFCLKTVYGSWIFPVFI  
KLTSVPTLTHNSLLAALFGGVIVGCGLGIVFWSDSSTGGTGIIQFLGKY  
TPISLGQGVLIDGLVTIVGFLAFDSDTVMFSIIGLITISYIINAIQTGFTTLST  
VLIVSQEHQKIKTYINTVADRGVTEIPVKGGYSGTNQIMLMTTIAGYEFA  
KLQEAIAEIDETAFTVTPTSQASGRGFSLQKNHGRLDEDILMPM\*

ID-50

Clone 99

ATGAAAGAAAAACAGTCGAAAAGGCTTATTTATATACTACTGATTGTTCCCATTAT  
CTTTATAAGTGTTTTTACATACAGTATTAGCCAGCCTTCTAACTACTTCCACCAAA  
AGAATTAGTTATTCTAAGTCCAAATAGTCAAGCCATTTTAACAGGAACGATTCCAG  
CTTTTGAGGAAAAATACGGTATAAAAGTTAAGCTTATTCAAGGTGGGACAGGGCA  
ACTAATAGATAGATTAAGTAAGGAGGGTAAGCAGTTGAAGGCGGATATTTCTTTG  
GAGGAAATTATACGCAATTTGAAAGTCATAAGGCATTGTTTGAGTCTTACGTATCA  
\_\_\_\_\_

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

AAGAATGTTTCATACTGTTATTCCAGACTATATCCATCCGAGTGATACGGCGACACC  
TTATACTATAAATGGGAGTGCTTTGATTGTAAATAACGAATTAGCTAAGGGACTTA  
CCATCAAGAGTTATGAAGATTTATTACAGCCTTCCTTAAAAGGTAAAATTGCCTTT  
GCAGATCCTCTAGAGTCGACCTGCAAGCATGCAAGCTTGGCGTAA

MKEKQSKRLIYILLIVPIIFISVFTYSISQPSKLLPPKELVILSPNSQAILTGTPAFEEKYGI  
KVKLIQGGTGQLIDRLSKEGKQLKADIFFGGNYTQFESHKALFESYVSKNVHTVIPDYI  
HPSDTATPYTINGSVLIVNNELAKGLTIKSYEDLLQPSLKGKIAFADPLESTCKHASLA

ID-51

Clone 103

CCTCCTATCAAATGATGACAAACGTGAGAGGTACATGGAACAAATGCTCTTTAAAA  
TTGAAAATGCAACCTGGCAGCGTGTGGTAAGAGCACTTTATCGTAAATACAATAAG  
GAATTTTTTACATATCCAGCCGCCAAAACAAACCACCGCTTTTGAATCAGGATT  
GGCATATCACACGGCAACAATGGTTCGTTTGGCAGATAGTATCGGAGATATCTATC  
CAGAACTTAATAAAAAGTTTGATGTTTGGCTGGTATTATGCTACATGATTTAGCCAAG  
GTCATAGAGTTATCGGGTCCTGATAATACAGAAATATACTATTTCGAGGTAATCTTAT  
CGGTCATATTTCACTTATTGATGAGGAATTAA

LLSNDDKRERYMEQMLFKIENATWQRVVRALYRKYNKEFFTYPAAKTNHHAFESGL  
AYHTATMVRLADSIGDIYPELNKSLMFAGIMLHDLAKVIELSGPDNTEYTRGNLIGHIS  
LIDEEL

ID-52

Clone 104

ATGAAAAAAAAATAAAATTATCCGATTCAGTTTAGTTGGTGTCTACTT  
GCGATACTATGCTTTAGTCTTTTTGCTTTATTGAAGCCTAACAGTCAA  
CAATCATCATCTCAAAAGTTGAGGAATGAGGATATAAAAAAGACATC  
CTCTCAAAAAAGAAATAAGAAATTACGATTACCAGCTGTATCATCAA  
AAGATTGGAACCTTGATTTTGGTCAATCGTGACCATAAACATGAAGAA  
TTAAGTCCAGATGTGGTGCCTGTTGAAAATATTTATTTGGATAAACGT  
ATTACGAAGCAAGCTACTCAGTTTTTAGAGGCTGCTAGAGCAATTGA  
TTCACGAGAACATTTAATTTCCGGGTATCGTAGTGTTGCCTATCAGGA  
GAAGTTGTTCAATTCTTATGTTACTCAAGAGATGACTAGTAACCCTAA  
TTTGACGAGGGGACAAGCAGAAAAGTTGGTAAAACTTACTCTCAGC  
CTGCAGGTGCTAGTGAACACCAGACTGGATTAGCGATGGATATGAGT  
ACTGTAGATTCTTTGAATGAGAGCGATCCTAGAGTAGTCAGTCAGTT  
GAAAAAGATAGCTCCACAATATGGTTTTGTCTTACGGTTTCCGGATG  
GTAAACACAGCAGAAACAGGGGTAGGTTATGAAGATTGGCATTACCG

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

CTATGTTGGGGTAGAGTCTGCAAAATATATGGTCAAACATCATTTAA  
CATTAGAAGAATACATAACTTTATTAAAGGAGAATAACCAATGA

MKKNKIIRFSLVGVLLAILCFSLFALLKPNSQQSSSQKL RNEDIKKTSSQK  
RNKKLRLPAVSSKDWNLILVNRD HKHEELSPDVVPVENIYLDKRITKQA  
TQFLEAARAIDSREHLISGYRSVAYQEKL FNSYVTQEMTSNP NLTRGQA  
EKL VKTYSQPAGASEHQTGLAMDMSTVDSL NESDPRVVSQ LKKIAPQY  
GFVLRFPDGKTAETGVGYEDWHYRYVGVESAKY MVKHHLTLEEYITLL  
KENNQ\*

ID- 53

Clone 106

CTGTTATGTGGATTTCTTCCATCAATTCCTGTGTCTAATTCCGGGGGGG  
TATGGTATAATAACAGTTATGAAAAATAAAAAAATCTTATTTGGGAC  
TGGCCTTGCTGGTGTGGGTTTACTGGCAGCTGCTGGTTATAACCCTAAC  
TAAAAAAGTAACAGATTATAAACGTCAGCAAATCACTCAGACCTTAA  
GAGAACTTTTTAGTCAGATGGGTGATATTCAGGTATTTTATTTAATG  
AATTTGAATCTGATATTAATAATGACCAGTGGTGGTCTTGTCTTGGAA  
GATGGCAGAATTTTCGAATTCATTTATCGTCAAGGTGTTCTTGATTAT  
GTGGAGGTGAGCAAATGA

LLCGFLPSIPVSNSGGYGHTVMKNKKILFGTGLAGVGLLAAAGYTLTKK  
VTDYKRQQITQTLRELF SQMGDIQVFYFNEFESDIKMTSGGLVLEDGRIF  
EFIYRQGVLDYVEVSK\*

ID-54

Clone 108

ATGTATCAAAC TCAGACAAATAAGGAAAAATTTGTTTTATTTTGA AATTATTTATC  
CCAGTATTGATTTATCAATTTGCTAATTTTTCAGCTACTTTTATTGATTCGGTTATGA  
CTGGACAGTATAGTCAGCTACATTTGGCAGGTGTGTCAACTGCTAGTAATTTATGG  
ACTCCGTTTTTTCGCTTTATTAGTAGGTATGATTTTCAGCATTAGTACCAGTAGTTGGT  
CAACATTTGGGTAGAGGAAATAAAGAACAAATTCGCACAGAATTTTCATCAATTTCT  
ATATTTAGGTTTGATACTGTCCTTAA

MYQTQTNKEKFVFLKLFIPVLIYQFANFSATFIDSVMTGQYSQLHLAGVSTASNLWTP  
FFALLVGMISALVPVVGQHLGRGNKEQIRTEFHQFLYLGLILSL

ID-55

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

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Clone 112

CTGCTCTTTTTAGCTAACTTTTCTAATTTATGGTATAATTGTATGGATT  
GTTTAGCTAGAATGGAGAAGATGATGCAAGATGTTTTTCATTATAGGA  
AGTAGAGGGTTGCCAGCTCGTTACGGTGGTTTTGAAACTTTTGTTTCA  
GAATTGATTAATCATCAAAAAAGTTCCGACATAAAATAACCATGTTGC  
ATGCCTTAGTGATAAAGAACATCATACTCATTTTAACTTTGCTGACGC  
TGATTGTTTTACTATAAATCCTCCCCAATTAGGGCCAGCACGTGTGAT  
TGCTTATGATATTATGGCCATTAATTATGCCCTTGACTTGGTTAAGAC  
ACATGATTTAAAAGAGCCTATTTTTTATATTTTAGGAAATACAATTGG  
TGCCTTTATTTGGCATTTTGCCAATAAAATACATAAAGTCGGTGGCTT  
ATTGTATGTTAATCCGGATGGTTTAGAGTGGAAGCGATCAAAGTGGT  
CTCGTCCACACAGCGTTATTTAAAATACGCCGAAAAATGTATGACT  
AAAAATGCAGACCTAATTATTTCTGATAATATTGGTATTGAAAATTA  
CATTCAATCTACCTACTCTAATGTGAAGACAAGGTTTCATTGCTTACGG  
TACAGAGATTAATTCTAGGAAATTATCGTCAGATGATCCACGTGTCA  
AACAGTTGTTTAAAAAATGGAATATTAAGTCTAAGGGTTACTATCTA  
ATCGTTGGTCGATTTGTCCCTGAAAACAATTATGAAACGGCTATTAG  
GGAGTTCATGGCTTCAGATACTAAGCGTGATTTAGTTATTATCTGTAA  
CCATCAAAATAACCCCTACTTTGAAAAGTTGTCCTTAAAGACAAACC  
TTCAACAAGATAAAAGAGTTAAGTTTGTAGGTACGCTCTATGAAAAA  
GATCTGCTGGATTATGTTTCGTCAACAAGCCTTTGCTTATATTCATGGG  
CATGAAGTTGGCGGTACTAATCCAGGACTGCTTGAGGCTTTAGCTAA  
TACTGATTTGAATCTTGTTCTAGATGTTGATTTCAACAAATCAGTAGC  
AGGTCTCTCAAGTTTTTACTGGACTAAAAAAGAGGGGGATTAGCTA  
AGCTT

MLFLANFSNLWYNCMDCLARMEKMMQDVFIIGSRGLPARYGGFETFVS  
ELINHQKSSDIKYHVACLSDKHHHTFNFADADCFTINPPQLGPARIAY  
DIMAINYALDLVKTHDLKEPIFYILGNTIGAFIWHFANKIHKVGGLLYVN  
PDGLEWKRSKWSRPTQRYLKYAEKCMTKNADLIISDNIGIENYIQSTYSN  
VKTRFIAYGTEINSRKLSSDDPRVKQLFKKWNKSKGYYLIVGRFVPENN  
YETAIREFMASDTKRDLVIICNHQNNPYFEKLSLKTNLQQDKRVKFGVT  
LYEKDLLDYVRQQAFAYIHGHEVGGTNPGLEALANTDLNLVLDVDFN  
KSVAGLSSFYWTKKEGDLAKL

ID-56

## Clone 120

TTGAGGAGTAATATGGTAAAGACAGCAGTTTTAATGGCGACATACAA  
TGGCGAAAAATTTATATCTGAACAACTTGATTCAATTCGCCAACAGA  
CATTAAAACCAGATTATGTATTATTGAGGGATGATTGTTCAACGGAT  
GAAACAGTCAATGTCGTCAATAACTATATCGCAAAACATGAGTTAGA

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FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

AGGCTGGAAAATTGTTAAAAACGACAAAACTTAGGCTGGCGTTTAA  
ATTTTCGTCAATTACTTATTGATGTGTTAGCCTATGAGGTTGACTATG  
TCTTTTTTAGTGATCAAGATGATATTTGGTATCTTGATAAAAAACGAAC  
GACAGTTTGCCATTATGTCAGATAACCCTCAAATTGAGGTTTTGAGTG  
CAGACGTTGATATCAAAACGATGTCTACAGAAGCCAGTGTTCCACAT  
TTTCTAACTTTTTCTTCTAGTGATAGAATCAGTCAGTATCCTAAAGTA  
TATGATTATCAAACATTCCGTCCTGGATGGACCATTGCTATGAAGAG  
AGATTTTGCGCAAGCTATCGCTTGA

MRSNMVKTAVLMATYNGEKFISEQLDSIRQQTLKPDYVLLRDDCSTDET  
VNVVNNYIAKHELEGWKIVKNDKNLGWRLNFRQLLIDVLAYEVDYVFF  
SDQDDIWYLDKNERQFAIMSDNPQIEVLSADVDIKTMSTEASVPHFLTFS  
SSDRISQYPKVYDYQTFRPGWTIAMKRDFQAIA\*

ID-57

Clone 123

GTGATTATGGATAAGTCTATTCCTAAAGCAACTGCTAAACGTTTATCA  
CTGTACTACCGTATTTTTAAACGTTTAAATACTGATGGCATCGAAAAA  
GCTAGTTCCAAACAAATTGCAGATGCCCTAGGTATCGATTCTGCTACT  
GTTTCGACGTGATTTTTCTTATTTTGGTGAAGTAGGACGCCGTGGTTTT  
GGTTATGATGTCAAAAAACTTATGAAGTTCTTTGCAGAAATATTGAA  
CGATCATTCTACAACAAATGTTATGCTGGTGGGGTGTGGAAATATCG  
GTAGAGCTCTCTTGCATTATCGTTTCCACGATCGCAATAAAATGCAA  
ATTTCAATGGCTTTTGATTAGATAGCAATGATTTAGTTGGTAAAACA  
ACCGAGGATGGAATTCCTGTCTACGGTATTTTCGACTATCAATGACCA  
TTTAATAGATAGTGATATTGAAACTGCTATCCTAACAGTACCTAGTAC  
AGAAGCCCAAGAAGTTGCTGACATCTTAGTCAAAGCAGGTATAAAA  
GGCATCTTGAGTTTTTCTCCAGTTCATTTAACATTACCAAAAGATATC  
ATTGTTCAGTATGTAGATTTAACAAGCGAATTACAACTTTACTTTAT  
TTCATGAACCAGCAGCGATAA

MIMDKSIPKATAKRLSLYYRIFKRFNTDGIEKASSKQIADALGIDSATVRR  
DFSYFGELGRRFGYDVKKLMNFFAEILNDHSTTNVMLVGCNGNIGRALL  
HYRFHDRNKMQISMAFDLDSNDLVGKTTEGIPVYGISTINDHLIDSDIE  
TAILTVPSTEAQEVADILVKAGIKGILSFSPVHLTLPKDIIVQYVDLTSELQ  
TLLYFMNQQR\*

ID-58

Clone 125

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**FIG. 1** CONT'D**SUBSTITUTE SHEET (RULE 26)**

ATGGGTGCTAAAGGAGCAGATGTCATTCTCGTTTTATCACACTCTGGCATTGGAGA  
TGATCGATATGAAGAAGGTGAAGAAAACGTTGGCTATCAAATTGCCAGCATCAAG  
GGAGTGGATGCCGTTGTTACGGGACACTCACACGCTGAATTTCCATCAGGTAACGG  
TACTGGCTTCTATGAAAAATACACTGGAGTTGATGGTATCAATGGAAAAATAAATG  
GAACACCTGTTACAATGGCAGGCAAGTACGGGGATCACCTTGGTATTATTGATTTA  
GGACTTAGTTATACTAATGGAAAAATGGCAAGTCTCCGAAAGCAGTGCTAAAATCC  
GTAAAATTGATATGAACTCAACAACCTGCTGACGAGCGTATCATTGCATTGGCTAAG  
GAAGCACACGATGGCACTATCAACTATGTTTCGCCAACAAGTAGGTACAACAACCTG  
CGCCAATTACAAGTTACTTTGCACTAGTTAA

MGAKGADVILVLSHSGIGDDRYEEGEENVGYQIASIKGVDVVTGHSHAEPSPGNGTG  
FYEKYTGVDGINGKINGTPVTMAGKYGDHLGIIDLGLSYTNGKWQVSESSAKIRKIDM  
NSTTADERIILAKEAHDGTINYVRQQVGTITAPITSYFALV

ID-59

Clone 135

TTGTCAATAAGGTTTCAAATCAGCTTGAAATATGATAAAATAAAACAGATTGTAAG  
TGACTGTTTAAGCTTGTTTTTCAGAGAGGTTTTTATGAATACAAACACAATAAAAA  
AGGTTGTAGCGACTGGAATTGGAGCTGCACTTTTTATCATTATAGGTATGCTAGTT  
AA

MSIRFQISLKYDKIKQIVSDCLSLFFREVMNTNTIKKVVATGIGAALFIIIGMLV

ID-60

Clone 145

ATGAAACATTTAAAATTTCAATCGGTCTTCGACATTATTGGTCCTGTTATGATTGGA  
CCATCAAGTAGTCATACTGCAGGAGCTGTCCGCATTGGTAAAGTTGTCCATTCTAT  
TTTTGGTGAACCTAGTGAAGTAACCTTTCATTTATACAATTCTTTTGCTAAACTTA  
CCAAGGACACGGTACTGATAAAGCATTGGTTGCAGGGATTCTAGGAATGGATACA  
GATAATCCAGATATTAA

MKHLKFQSVFDIIGPVMIGPSSHTAGAVRIGKVVHSIFGEPSEVTFHLYNSFAKTYQG  
HGTDKALVAGILGMDTDNPD

ID-61

Clone 147

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FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

GTGTCAGAAAGGTGTTTTAATGTTTCTAAAAGAAGATGACGTAGAGACTTTTCTTCA  
TATCCTGACAAATTCATTTAGCCAATTTATGGCACAATTTGATTTGTGTCATAAGGA  
AATGATTAA

ID-62

Clone 150

ATGACCTACAAAGATTACACAGGTTTAGATCGGACTGAACTTTTGAGTAAAGTGCG  
TCATATGATGTCCGACAAACGTTTTAA

MTYKDYTGLDRTTELLSKVRHMMMSDKRF

ID-63

Clone S2

CTGAGTTGGGTCTTGGAACGGTCCTGTCAATCATACTAGCTATCAAGGAGACTAA  
AATGTATTTAGAACAATAAAAGAGGTAAATCCTTTAA

MSWVLETVLSIILAIIKETKMYLEQLKEVNPL

ID-67

Clone 3-40

GTGAAAAAAAAAATTAGTCTCATCACTTCTAAAGTGTTCTCTAATCATT  
ATTGTTAGCTTTGCTGGTGGAGCATTTGCTAGTTTTGTCATGAATCAT  
AATGACAATATTCCAAATGGTGGTGTCACTAAACTAGTAAAGTAAA  
TTATAATAACATAACGCCTACAACAAAAGCTGTTAAAAAGGTACAAA  
ATAGTGTTGTTTCTGTTATCAATTATAACAACAAGAGAGTCGTTCTG  
ACCTATCAGACTTCTATAGTCATTTTTTTGGTAATCAGGGGGGCAACA  
CTGATAAGGGCTTACAAGTTTACGGTGAAGGCTCTGGAGTCATCTAT  
AAAAAAGATGGTAAAAATGCCTATGTTGTCACTAATAACCACGTCAT  
TGATGGGGCTAAACAAATTGAAATTCAACTAGCTGATGGCTCAAAAG  
CAGTTGGGAACTTGTTGGGTCAGATACCTACTCTGATTTAGCCGTCG  
TCAAAATTCCATCAGATAAAGTTTCAAATATTGCAGAATTTGCTGATT  
CATCAAACTCAACATTGGTGAACTGCTATAGCGATCGGAAGCCCT  
CTTGGAAGTGAAGTATGCAAATCTGTAAGTCAAGGTATTGTATCTAGT  
TAAAAAAGAACTGTAACAATGACTAATGAAGAAGGACAAACAGTTT  
CTACAAATGCTATCCAGACGGATGCTGCTATCAATCCTGGTAATTCA  
GGTGGAGCACTTATCAATATTGAAGGACAGGTTATTGGAATTAATTC  
TAGTAAAATTTCTTCTACATCAAATCAAACCTCAGGACAATCGTCAG

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

GAAATAGCGTTGAAGGTATGGGATTTGCCATTCCTTCAAATGATGTT  
GTTAAGATTATCAATCAACTTGAGAGTAACGGACAAGTAGAGAGACC  
TGCTCTAGGTATTTCTATGGCTGGATTAAGTAATTTACCATCCGATGT  
TATTAGTAAACTGAAAATCCCAAGTAATGTTACTAATGGTATTGTAG  
TAGCATCTATCCAATCTGGCATGCCAGCTCAAGGCAAACCTAAAGAAA  
TACGATGTCATTACTAAAGTTGACGATAAAGAAGTAGCATCTCCAAG  
TGATTTACAAAGTTTACTCTATGGCCACCAGGTAGGGGATTCCATAA  
CAGTAACCTTTTATCGTGGTGAAAATAAACAAACAGTCACTATAAAA  
CTTACTAAAACCTAGTAAAGATTTAGCTAAACAACGAGCAAATAACTA  
A

MKKKL VSSLLKCSLIIIVSFAGGAFASFVMNHNDNIPNGGVTKTSKVNY  
NNITPTTKAVKKVQNSVVSVINYKQESRSDLSDFYSHFFGNQGGNTDK  
GLQVYGEKSGVYKKGDKNAYVVTNNHVIDGAKQIEIQLADGSKAVGK  
LVGSDTYSDLA VVKIPSDKVSNAIEFADSSKLNIGETAIAIGSPLGTEYAN  
SVTQGI VSSLKRTVTMTNEEGQTVSTNAIQTDAAINPGNSGGALINIEGQ  
VIGINSSKISSTSNQTSQGSSGNSVEGMGFAIPSN DVVKIINQLESNGQVE  
RPALGISMAGLSNLPSDVISKLKIPSNVTNGIVVASIQSGMPAQGKLKKY  
DVITKVDDKEVASPSDLQSLLYGHQVGDSITVTFYRGENKQTVTIKLT KT  
SKDLAKQRANN\*

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Clone 3-30

ATGTTAAAATGGTATACAAACAAAGGAGGGAGGATGATAATGAAGA  
AATGTTTTTTGGCTATTTGTTTAGCTCTTAGTTTTTTTATGGTTTTAGT  
TCAAGCAGATGAGGTGGACTATAACATTCCTCATTATGAGGGTAATC  
TAACTATT CACAATGATAATAGTGCTGATTTTACAGAGAAGGTTACTT  
ACCAATTTGATTCGTCCTATAATGGACAGTATGTCACGTTAGGTACG  
GCGGGTAAGTTATCTGACAATTTTGATATTAATAATAAGCCACAGGT  
TGAAGTTTCAATTAATGGTAAAGTAAGGAAAGTTAGTTACCAGATAG  
AAGATTTGGAGGATGGCTACCGTTTGAAAGTGTTTAATGGTGGTGAA  
GCAGGTGATACTGTTAAAGTCAATGTTTCAGTGGAACTAAAAAATGT  
TCTATTTATGCATAAGGATGTTGGTGAACCTTAAGTGGATTCCTATTAG  
CGACTGGGATAAAACGTTAGAGAAAGTAGATTTTTGGATATCAACTG  
ACAAAAAGGTTGCTCTTTCTCGTCTTTGGGGGCACTTGGGTATCTTA  
AACTCCTCCTAAAATAAGACAAAATAATAATCGTTACCATTTGACA  
GCTTTTAATGTAAACAAACGATTAGAATTTTATGGTTATTGGGATAG  
ATCTTATTTTAATCTACCTACAAACAGTAAAAATAATTACAAGAAAA  
AAATTGAACATCAAGAGAAGATAATAGAGCGTCATGGTTTTATCCTA  
AGTTTCTTGTTAAGGATATTATTACCTTCATTCTTTATTATTGTGACAC  
TATTCATCTCAATTAGGGTGTTCTCTGTTTAGAAAAAAGTTAATAAAT

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

ACGGGCAATTCCCTAAGGATCATCATTTATATGAAGCACCTGAGGAC  
CTTTCACCATTAGAGTTAACTCAAAGCATTTATAGTATGAGCTTTAAA  
AATTTTCAAGATGAGGAGAAGAAAACCTCACCTTATCAGTCAAGAACA  
ACTCATACAGTCAATTCTATTAGACTTGATTGATAGAAAAGTATTGA  
ATTATGATGATAACTTGTTATCTCTAGCTAACTTAGATAGAGCTTCTG  
ATGCAGAAATAGATTTTATAGAGTTTGCTTTTGCGGATTCTACGAGTT  
TGAAGCCAGATCAACTCTTTTCTAATTACCAATTTAGTTATAAAGAAA  
CACTACGTGAACTGAAAAAGCAGCACAAAGGCTTCAGATCTGCAAAAT  
CAAATGAGACGCCGAGGAAGTAATGCCTTATCAAGAATTACGCGTCT  
CACAAGGTTGATTTCTAAAGACAATATAAACTCTCTTAGAAGAAAGG  
GAATTTTCATCCCCTTATCGTAAAATGTCTTCAGAAGAGTCTAAAGAA  
TTATCTAGGTTAAAAAGATTCAGTTACCTATCACCTCTTATTTCTTTTG  
TTGTTATAATTTATACGCTTTTTTTAAATTATTTTACCTATTTCTGTAT  
CTATCTCTTATTGTTTGGTGTTATCCTGTTGTTGAATAAAATCATTTTT  
ATGATGACAAGAAAAATAAGTAACGGTTATATTGTAAGTGAAGATGG  
AGCAAGTCGTGTCTACCAATGGACTAGTTTTAGGAACATGCTAAGGG  
ATATCAAATCGTTTGATCGTTCAGAGTTAGAAAGTATCGTATTATGG  
AATCGAATATTGGTTTACGCTACTTTATTTCGGCTACGCTGACCGTGTT  
GAGAAAGTACTCAGAGTGAACCAAATAGATATTCCAGAAAGATTTGC  
AAACATTGATAGTCATCGATTTGCGATTTTCAGTCAATCAATCTAGTAA  
TCATTTTTCAACGATAACTGAAGATGTTAGTCACGCTTCTAATTTTAG  
TGTTAATTCAGGCGGTTCTTCAGGTGGTTTCTCAGGCGGCGGAGGCG  
GCGGAGGTGGCGGTGCCTTCTAA

MLKWYTNKGGRMIMKKCFLAICLALSFFMVSVQADEVDYNIPHYEGNL  
TIHNDNSADFTEKVITYQFDSSYNGQYVTLGTAGKLSDNFDINNKPQVEV  
SINGKVRKVSQIEDLEDGYRLKVFNGGEAGDTVKNVQWKLKNVLF  
MHKDVGELNWIPISDWKLTLEKVDWFISTDKKVALSRLWGHLGYLKTP  
PKIRQNNNRYHLTAFNVNKRLEFHGYWDRSYFNLPTNSKNNYKKKIEH  
QEKIHERHGFILSFLLRILLPSFFIIVTLFISIRVFLFRKKVNKYGQFPKDHHL  
YEAPEDLSPLELTQSIYSMSFKNFQDEEKKTHLISQEQLIQSILLDLDRKV  
LNYDDNLLSLANLDRASDAEIDFIEFAFADSTSLKPDQLFSNYQFSYKET  
LRELKKQHKASDLQNQMRRRGSNALSRTLRTRLISKDNINSLRRKGISS  
PYRKMSSEESKELSRCLKRFSYLSPLISFVVIYTLFLNYFTYFCIYLLLFYVI  
LLLNKIIIFMMTRKISNGYIVTEDGASRVYQWTSFRNMLRDIKSFRSELE  
SIVLWNRILVYATLFGYADRVEKVLRVNQIDIPERFANIDSHRFAISVNQS  
SNHFSTITEDVSHASNFSVNSGGSSGGFSGGGGGGGGAF\*

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Clone 3-38

ATGATGATTGTGAATAATGGTTATCTAGAAGGGAGAAAAATGAAAA  
AGAGACAAAAAATATGGAGAGGGTTATCAGTTACTTTACTAATCCTG

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

TCCCAAATTCCATTTGGTATATTGGTACAAGGTGAAACCCAAGATAC  
CAATCAAGCACTTGGAAAAGTAATTGTTAAAAAACGGGAGACAAT  
GCTACACCATTAGGCAAAGCGACTTTTGTGTTAAAAAATGACAATGA  
TAAGTCAGAAACAAGTCACGAAACGGTAGAGGGTTCTGGAGAAGCA  
ACCTTTGAAAACATAAAACCTGGAGACTACACATTAAGAGAAGAAA  
CAGCACCAATTGGTTATAAAAAAACTGATAAAACCTGGAAAGTTAAA  
GTTGCAGATAACGGAGCAACAATAATCGAGGGTATGGATGCAGATA  
AAGCAGAGAAACGAAAAGAAGTTTTGAATGCCCAATATCCAAAATC  
AGCTATTTATGAGGATACAAAAGAAAATTACCCATTAGTTAATGTAG  
AGGGTTCCAAAGTTGGTGAACAATACAAAGCATTGAATCCAATAAAT  
GGAAAAGATGGTCGAAGAGAGATTGCTGAAGGTTGGTTATCAAAAA  
AAAATCCAGGGGTCAATGATCTCGATAAGAATAAATATAAAATTGAA  
TTAACTGTTGAGGGTAAAACCACTGTTGAAACGAAAGAACTTAATCA  
ACCACTAGATGTCGTTGTGCTATTAGATAATTCAAATAGTATGAATA  
ATGAAAGAGCCAATAATTCTCAAAGAGCATTAAAAGCTGGGGAAGC  
AGTTGAAAAGCTGATTGATAAAATTACATCAAATAAAGACAATAGA  
GTAGCTCTTGTGACATATGCCTCAACCATTTTGTATGGTACTGAAGCG  
ACCGTATCAAAGGGAGTTGCCGATCAAAATGGTAAAGCGCTGAATG  
ATAGTGTATCATGGGATTATCATAAACTACTTTTACAGCAACTACA  
CATAATTACAGTTATTTAAATTTAACAAATGATGCTAACGAAGTTAA  
TATTCTAAAGTCAAGAATTCCAAAGGAAGCGGAGCATATAAATGGG  
GATCGCACGCTCTATCAATTTGGTGCGACATTTACTCAAAAAGCTCTA  
ATGAAAGCAAATGAAATTTTAGAGACACAAAGTTCTAATGCTAGAAA  
AAAACCTATTTTTTACGTAAGTATGGTGTCCCTACGATGTCTTATGC  
CATAAATTTTAATCCTTATATATCAACATCTTACCAAAACCAGTTTAA  
TTCTTTTTTTAAATAAAATACCAGATAGAAGTGGTATTCTCCAAGAGG  
ATTTTATAATCAATGGTGATGATTATCAAATAGTAAAAGGAGATGGA  
GAGAGTTTTAACTGTTTTCGGATAGAAAAGTTCCTGTTACTGGAGG  
AACGACACAAGCAGCTTATCGAGTACCGCAAAATCAACTCTCTGTAA  
TGAGTAATGAGGGATATGCAATTAATAGTGGATATATTTATCTCTATT  
GGAGAGATTACAACCTGGGTCTATCCATTTGATCCTAAGACAAAGAAA  
GTTTCTGCAACGAAACAAATCAAACTCATGGTGAGCCAACAACATT  
ATACTTTAATGGAAATATAAGACCTAAAGGTTATGACATTTTACTGT  
TGGGATTGGTGTAACCGGAGATCCTGGTGCAACTCCTCTTGAAGCTG  
AGAAATTTATGCAATCAATATCAAGTAAAACAGAAAATTATACTAAT  
GTTGATGATACAAATAAAATTTATGATGAGCTAAATAAATACTTTAA  
ACAATTGTTGAGGAAAAACATTCTATTGTTGATGGAAATGTGACTG  
ATCCTATGGGAGAGATGATTGAATTCCAATTAATAAATGGTCAAAGT  
TTTACACATGATGATTACGTTTTGGTTGGAAATGATGGCAGTCAATTA  
AAAAATGGTGTGGCTCTTGGTGGACCAACAGTGATGGGGGAATTTT  
AAAAGATGTTACAGTGACTTATGATAAGACATCTCAAACCATCAAAA  
TCAATCATTTGAACTTAGGAAGTGGACAAAAGTAGTTCTTACCTAT  
GATGTACGTTTAAAAGATAACTATATAAGTAACAAATTTTACAATAC  
AAATAATCGTACAACGCTAAGTCCGAAGAGTGAAAAAGAACCAAT

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

ACTATTCGTGATTTCCTCAATTCCCAAATTCGTGATGTTTCGTGAGTTT  
CCGGTACTAACCATCAGTAATCAGAAGAAAATGGGTGAGGTTGAATT  
TATTAAAGTTAATAAAGACAAACATTCAGAATCGCTTTTGGGAGCTA  
AGTTTCAACTTCAGATAGAAAAAGATTTTTCTGGGTATAAGCAATTT  
GTTCCAGAGGGAAGTGATGTTACAACAAAGAATGATGGTAAAATTTA  
TTTTAAAGCACTTCAAGATGGTAACTATAAATTATATGAAATTTCAA  
GTCCAGATGGCTATATAGAGGTTAAAACGAAACCTGTTGTGACATTT  
ACAATTCAAAATGGAGAAGTTACGAACCTGAAAGCAGATCCAAATG  
CTAATAAAAAATCAAATCGGGTATCTTGAAGGAAATGGTAAACATCTT  
ATTACCAACACTCCCAAACGCCACCAGGTGTTTTCTCTAAAACAGG  
GGGAATTGGTACAATTGTCTATATATTAGTTGGTTCTACTTTTATGAT  
ACTTACCATTTGTTCTTTCCGTCGTAAACAATTGTAA

MMIVNNGYLEGRKMKKRQKIWRGLSVTLLILSQIPFGILVQGETQDTNQ  
ALGKVIVKKTGDNATPLGKATFVLKNDNDKSETSHETVEGSGEATFENI  
KPGDYTLREETAPIGYKKTDKTWKVKVADNGATIIEGMDADKAERKE  
VLNAQYPKSAIYEDTKENYPLVNVEGSKVGEQYKALNPINGKDGREIA  
EGWLSKKNPGVNDLDKNKYKIELTVEGKTTVETKELNQPLDVVLLDN  
SNSMNNERANNSORALKAGEAVEKLIDKITSNKDNRVALVTYASTIFDG  
TEATVSKGVADQNGKALNDSVSWDYHKTTFTATTHNYSYLNLTNDAN  
EVNILKSRIPKEAEHINGDRTLYQFGATFTQKALMKANEILETQSSNARK  
KLIFHVTGDGVPTMSYAINFNPIYSTSYQNQFNSFLNKIPDRSGILOEDFIIN  
GDDYQIVKGDGESFKLFSDRKVPVTGGTTQAAYRVPQNQLSVMSNEGY  
AINSGYTYLYWRDYNWVYPFDPKTKKVSATKQIKTHGEPTTLYFNGNIR  
PKGYPDFTVGIGVNGDPGATPLEAEKFMQSISSKTENYTNVDDTNKIYDE  
LNKYFKTIVEEKHSIVDGNVTDPMGEMIEFQLKNGQSFTHDDYVLVGND  
GSQKNGVALGGPNSDGGILKDVTVTYDKTSQTIKINHLNLGSGQKVVL  
TYDVRLKDNYISNKFYNTNNRTTLPKSEKEPNTIRDFPIPKIRDVREFPV  
LTISNQKKMGEVEFIKVNKDKHSESLGAKFQLQIEKDFSGYKQFVPEGS  
DVTTKNDGKIYFKALQDGNKYLYEISSPDGYIEVKTKPVVTFITQNGEVT  
NLKADPNANKNQIGYLEGNGKHLITNTPKRPPGVFPKTGGIGTIVYILVG  
STFMILTICSFRRKQL\*

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Clone 141

ATGAATAGAAAAGTTGAGGAAAAAATGGCTGGGAATCGTAATAACG  
ATATGAATGTCTATTGTTCAATTTTGTGGCAAAAGCCAAGATGAAGTA  
AAAAAAATTATTGCAGGTAATGGTGTTCATTTGTAATGAATGTGTG  
GCCTTATCACAAGAAATTATTAAGGAAGAATTAGCTGAGGAAGTACT

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

GGCTCATTTAGCAGAAGTACCAAAACCTAAGGAACTATTAGAAATAT  
TAAATCAATATGTTGTAGGGCAAGATCGTGCTAAACGTGCTTTAGCA  
GTTGCTGTCTACAATCATTACAAGCGTGTTAGTTATACCGAGAGTAGT  
GACGATGATGTAGATTTGCAAAAATCCAACATTTTGATGATTGGTCC  
AACTGGCTCAGGAAAAACCTTCTTAGCACAAACACTGGCTAAAAGCC  
TTAATGTACCGTTTGCTATTGCAGATGCGACTTCATTGACCGAAGCAG  
GATACGTTGGAGAAGATGTTGAGAATATTCTTCTTAAATTGATTCAA  
GCTGCTGATTATAATGTCGAACGTGCTGAGCGTGGTATTATCTACGTT  
GATGAAATAGATAAAATTGCTAAGAAAGGCGAAAATGTTTCTATCAC  
ACGTGATGTGTCTGGTGAAGGTGTACAGCAAGCCCTTCTTAAATTA  
TTGAGGGTACGGTAGCAAGTGTTCCCCCACAGGGTGGGCGTAAACAT  
CCTAACCAAGAAATGATTCAAATTAATACCAAGAACATCCTTTTTATT  
GTCGGTGGTGCTTTTGATGGTATTGAAGACCTTGTGAAGCAACGTTTA  
GGCGAAAAAGTTATTGGTTTTGGACAGACAAGCCGTAAAATTGATGA  
CAACGCTTCTTATATGCAAGAGATAATTTCTGAGGATATTCAAAAGT  
TTGGACTGATTCCAGAGTTTATTGGCCGTTTACCAGTAGTTGCAGCGT  
TAGAACTTCTTACTGCAGAAGATCTGGTTCGTATTCTGACAGAACCA  
CGCAATGCTTTGGTTAAACAATACCAACCTTATTATCTTATGATGGT  
GTAGAATTGGAATTTGACCAGGATGCTCTATTGGCTATCGCTGATAA  
GGCTATCGAGCGCAAGACTGGTGCACGTGGTTTACGTTCTATTATTG  
AAGAAACGATGCTTGATATCATGTTTGAAATTCCAAGCCAAGAAGAT  
GTAACAAAAGTTCGTATCACAAAGGCTGCTGTTGAGGGTACTGACAA  
GCCTGTTTTAGAGACGGCTTAG

MNRKVEEKMAGNRNNDMNVYCSFCGKSQDEVKKIAGNGVFICNECV  
ALSQEIKEELAEVLAHLAEVPKPKELLEILNQYVVGQDRAKRALAVA  
VYNHYKRVSYTESSDDVDLQKSNILMIGPTGSGKTFLAQTAKSLNVP  
FAIADATSLTEAGYVGEDVENILLKLIQAADYNVERAERGIIYVDEIDKIA  
KKGENVSITRDVSGEGVQQALLKIIEGTVASVPPQGGRKHPNQEMIQINT  
KNILFIVGGAFDGIEDLVKQRLGEKVIGFGQTSRKIDDNASYMQEIISED  
QKFGLIPEFIGRLPVVALELLTAEDLVRLTEPRNALVKQYQTLISYDG  
VELEFDQDALLAIADKAIERKTGARGLRSHIETMLDIMFEIPSQEDVTKV  
RITKAAVEGTDKPVLETA\*

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Clone 3-20

ATGAAAAGATTACATAAACTGTTTATAACCGTAATTGCTACATTAGG  
TATGTTGGGGGTAATGACCTTTGGTCTTCCAACGCAGCCGCAAAACG  
TAACGCCGATAGTACATGCTGATGTCAATTCATCTGTTGATACGAGC  
CAGGAATTTCAAAATAATTTAAAAAATGCTATTGGTAACCTACCATT  
TCAATATGTTAATGGTATTTATGAATTAAATAATAATCAGACAAATTT  
AAATGCTGATGTCAATGTAAAGCGTATGTTCAAATAACAATTGACA

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

ATCAACAAAGACTATCAACTGCTAATGCAATGCTTGATAGAACCATT  
CGTCAATATCAAAATCGCAGAGATACCACTCTTCCCGATGCAAATTG  
GAAACCATTAGGTTGGCATCAAGTAGCTACTAATGACCATTATGGGC  
ATGCAGTCGACAAGGGGCATTTAATTGCCTATGCTTTAGCTGGAAAT  
TTCAAAGGTTGGGATGCTTCCGTGTCAAATCCTCAAAATGTTGTCACA  
CAAACAGCTCATTCCAACCAATCAAATCAAAAAATCAATCGTGGACA  
AAATTATTATGAAAGCTTAGTTCGTAAGGCGGTTGACCAAAACAAAC  
GTGTTTCGTTACCGTGTAACCTCATTGTACCGTAATGATACTGATTTAG  
TTCCATTTGCAATGCACCTAGAAGCTAAATCACAAGATGGCACATTA  
GAATTTAATGTTGCTATTCCAAACACACAAGCATCATACACTATGGA  
TTATGCAACAGGAGAAATAACACTAAATTAA

MKRLHKLFIITVIATLGMLGVMTFGLPTQPQNVTPIVHADVNSSVDTSQE  
FQNNLKNAIGNLPFQYVNGIYELNNNQTNLNADVNVKAYVQNTIDNQQ  
RLSTANAMLDRTIRQYQNRDITLPDANWKPLGWHQVATNDHYGHAV  
DKGHLIAYALAGNFKGWDASVSNPQNVVTQTASHNQSNOQKINRGQNY  
YESLVRKAVDQNKRVRYRVTPLYRNDTDLVPFAMHLEAKSQDGTLEFN  
VAIPNTQASYTMDYATGEITLN\*

ID-72

Clone 13

ATGAAAAACTATCGAAAACTTATTGTACTACTACTTCTAATCTTTTTT  
GCCATTTTTATGGGAGCATATGCTTACACGCATATTGTTGAAAAAAG  
ATCCCTAACTAGCAATACTATTGAAAAAACTCTACCTGTGGTAAATC  
AGATTAAGCCTCAAACCATTAAGAATAACCAAAATTACTTAACCTAAG  
GTAGCTAAACGTAATGTTCTTCTGTAGACATTCCTCAGGCATTAAAT  
AATGAAAAGGTAGAAATTACTGCTACTGATGGCATGCAACATTAC  
TTGGAATGATAAAAAATAATCCTAAGCAAAAGGTTATCTTCTATGTTT  
ATGGAGGATCATATATCCATCAAGCTTCCGAATTACAATATATTTTTG  
TCAATAAACTAGCTAAAAAATTAGATGCAAAAGTTGTCTTTCCTATTT  
ACCCTAAAGCTCCTACATATAATTATAGTGATGCTATCCCCAAAATTA  
AAAAATTATACCAAAATACATTAGCTAGCGTCACATCTCACAAACAG  
ATTATCCTAGTAGGTGAAAGTGCAGGCGGAGGCCTTGCTTTAGGTAT  
TGCTGATAACCTTGACGAGCATATCAAACAACCAAAAGAAATTAT  
TTTAA

MKNYRKLIVLLLLIFFAIFMGAYAYTHIVEKRSLTSNTIEKTLPVVNQIKP  
QTIKEYQNYLTKVAKRNVLPVDIPQALNNEKVEITATDGMQFTFTWNDK  
NNPKQKVIFYVHGGSYIHQASELQYIFVNKLAKKLDKVVFPPIYPKAPT  
YNYSDAIPKIKKLYQNTLASVTSHKQIILVGESAGGGLALGIADNLARSIS  
NNQKKLF\*

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

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ID-73

Clone 2-19

TTGATTCTAATAACTTCCTATGGGATAATATCTTTATCACAAAAATTG  
AGGGAATTTATTATGAAGTTAAAACATATTGTCTTAGGATTAGCCTTA  
ACAACACTTTTAGGAGTCACATTTAGTAATCAAGAAGTTTCAGCAAG  
CTCAACTTCAAGTAAAGTTGTTAAAGTTGGTGTTATGACCTTTTCTGA  
CACTGAAAAAGCACGTTGGGATAAAATTGAAAAGCTAGTAGGTGAT  
AAAGCTAAAATCAAATTTACAGAATTTACAGATTATACACAACCAAA  
TCAAGCGACAGCCAATAAGGATGTGGATATTAATGCCTTTCAACATT  
ACAATTTCTTAGAAAACTGGAATAAGGAAAAATAAGAAAAACTTAATT  
CCACTTGAAAAGACTTACTTAGCTCCAATTCGTATCTATTCTGAGAAG  
GTAAAATCTCTTAAAAAATTGAAAAAAGGAGCCACTATTGCAATTCC  
AAATGATGCAACAAATGGTAGCCGTGCATTGTATGTCCTTCAGTCAG  
CAGGTTTAATCAAATTGAATGTTTCTGGTAAGAAGGTTGCAACAGTT  
GCTAATATCACATCTAATAAAAAAGGATATTAATATTCAGGAGTTAGA  
TGCGAGTCAAACACCACGTGCACTCAAAGATGTAGATGCAGCTATTA  
TTAATAATACATACATTGAGCAAGCTAATTTAAAACCTTCAGATGCT  
ATCTTTGTTGAGAAATCAGATAAAAATTCAAACAATGGATTAATAT  
CATTGCGGGACGTAAAAATTGAAAAAAGCAAAAGAACGCTAAAGCT  
ATCCAAGCTATCTTGGATGCTTATCACACAGATGAAGTGAAAAAAGT  
TATCAAAGATACTTCAGCTGATATTCCACAATGGTAA

MILITSYGIISLSQKLREFIMKLKHIVLGLALTTLLGVTFNSNQEVSSSTSS  
KVVKVGVMTFSDTEKARWDKIEKLVGDKAKIKFTEFTDYTQPNQATAN  
KDVDINAFQHYNFLENWKNENKKNLIPLEKTYLAPIRIYSEKVKSLKKL  
KKGATIAIPNDATNGSRALYVLQSAGLIKLVSGKKVATVANITSNKKDI  
NIQELDASQTPRALKDVDAAIINNTYIEQANLKPSDAIFVEKSDKNSKQW  
INILAGRKNWKKQKNAKAIQAILDAYHTDEVKKVIKDT SADIPQW\*

ID-74

Clone 3-6

ATGTCAAATCAATATGATTATATCGTTATTGGTGGAGGTAGTGCAGG  
CAGTGGTACCGCTAATAGGGCAGCCATGTATGGAGCAAAAGTCCTGT  
TAATTGAAGGTGGACAAGTAGGTGGAACCTTGTTAACTTAGGTTGT  
GTACCTAAGAAAATCATGTGGTATGGTGCACAAGTTTCTGAGACACT  
CCATAAGTATAGTTCAGGTTATGGTTTTGAAGCCAATAATCTTAGTTT  
TGATTTTACTACTCTAAAAGCTAATCGCGATGCTTACGTGCAGCGGTC  
TAGACAGTCGTATGCCGCTAATTTTGAGCGTAATGGGGTCGAAAAGA

---

FIG. 1 CONT'D

TTGATGGATTTGCTCGTTTTATTGATAACCATACTATTGAAGTGAATG  
GTCAGCAATATAAAGCTCCTCACATTACTATTGCAACAGGTGGACAC  
CCTCTTTACCCTGATATTATTGGAAGTGAACCTGGTGAGACTTCTGAT  
GATTTTTTTGGATGGGAGACCTTACCAAATTCTATATTGATTGTTGGG  
GCGGGCTATATCGCGGCAGAACTTGCTGGAGTGGTTAATGAATTAGG  
CGTTGAAACCCATCTTGCATTTAGAAAAGACCATATTCTACGCGGAT  
TTGATGACATGGTAACAAGTGAGGTTATGGCTGAAATGGAGAAATCA  
GGTATCTCTTTACATGCTAACCATGTACCTAAATCTCTTAAACGCGAT  
GAAGGTGGCAAGTTGATTTTTGAAGCTGAAAATGGGAAAACGCTTGT  
CGTTGATCGTGTAATATGGGCTATCGGCCGTGGACCAAATGTAGACA  
TGGGACTTGAAAATACCGATATTGTTTTAAATGATAAAGATTATATC  
AAAACAGATGAATTTGAGAATACTTCTGTAGATGGCGTGTATGCTAT  
TGGAGATGTTAATGGGAAAATTGCCTTGACACCGGTAGCAATTGCAG  
CAGGTCGTCGCTTATCAGAAAGACTTTTTAATCATAAAGATAACGAA  
AAATTAGATTACCATAATGTACCTTCAGTTATTTTTACTCACCTGTA  
ATTGGGACGGTAGGACTTTCAGAAGCAGCAGCTATCGAGCAATTTGG  
AAAAGATAATATCAAAGTCTATACATCAACTTTTACCTCTATGTATAC  
GGCTGTTACCAGTAATCGCCAAGCAGTTAAGATGAAGCTCATAACCC  
TAGGAAAAGAGGAAAAAGTTATTGGGCTTCATGGTGTGGTTATGGT  
ATTGATGAAATGATTCAAGGTTTTTCAGTTGCTATCAAAATGGGGGC  
TACTAAAGCAGACTTTGATGATACTGTTGCTATTCACCCAAGTGGATC  
TGAGGAATTTGTTACAATGCGCTAA

MSNQYDYIVIGGGSAGSGTANRAAMYGAKVLLIEGGQVGGTCVNLGC  
VPKIMWYGAQVSETLHKYSSGYGFEANNLSFDFTTLKANRDAYVQRS  
RQSYAANFERNGVEKIDGFARFIDNHTIEVNGQQYKAPHITIATGGHPLY  
PDIIGSELGETSDDFFGWETLPNSILIVGAGYIAAELAGVVNELGVETHLA  
FRKDHILRGFDDMTSEVMAEMEKSGISLHANHVPKSLKRDEGGKLIFE  
AENGKTLVVDRVIWAIGRGPVNDMGLENTDIVLNDKDYIKTDEFENTSV  
DGVYAIGDVNGKIALTPVALAAGRRLSERLFNHKDNEKLDYHNVPSVIF  
THPVIGTVGLSEAAAIEQFGKDNKVYTSTFTSMYTAVTSNRQAVKMKLI  
TLGKEEKVIGLHGVGYGIDEMIQGFSAIKMGATKADFDFTVAIHPTGS  
EEFVTMR\*

ID-75

Clone 3-51

ATGAGTATCAAAAAAAGTGTGATTGGTTTTTGCCTCGAAGCTGCAGC  
ATTATCAATGTTTGCTTGTGTAGACAGTAGTCAATCTGTTATGGCTGC

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

CGAGAAGGATAAAGTCGAAATTACGTGGTGGGCTTTTCCAACCTTTA  
CTCAAGAAAAGGCTAAGGATGGAGTAGGTACTTATGAGAAAAAAGT  
CATCAAGGCTTTTGAAAAGAAAAATCCTAATATAAAAGTAAACTAG  
AGACAATTGATTTACATCTGGACCTGAAAAAATCACTACAGCAATT  
GAAGCAGGGACAGCACCTGATGTGCTTTTTGATGCACCAGGGCGAAT  
TATTCAATATGGTAAAAATGGTAAATTAGCAGATTTGAATGATTTATT  
TACAGACCAATTTATTAAGGATGTCAATAATAAGAACATCATTCAAG  
CTTCTAAGTCTGGCGATAAAGCCTACATGTATCCAATAAGTTCTGCCC  
CATTTTATATGGCGTTCAATAAAAAAATGCTTAAAGATGCAGGAGTT  
TTGAAACTTGTAAGAAGGTTGGACTACTAGTGATTTTGAAAAAGT  
ACTAAAAGCACTAAAAAATAAAGGCTATACACCAGGTTTCATTCTTG  
CAAACGGGCAAGGAGGAGATCAAGGACCACGTGCATTTTTTGCTAAT  
CTTTATAGTGCTCCAATAACAGATAAAGAAGTAACAAAATATACCAC  
TGACACTAAAAATTCTGTAAAATCAATGAAAAAATAGTTGAATGGA  
TTAAGAAAGGCTACTTGATGAATGGGTCTCAGTATGATGGCTCAGCT  
GACATTCAAACTTCGCCAATGGACAACTGCTTTCCTATCCTATG  
GGCTCCAGCTCAACCAAAAACTCAAGCAAAATTATTAGAGTCAAGTA  
AAGTGGATTACCTTGAAGTGCCATTCCCATCAGAAGATGGAAAACCA  
GATTTAGAATACCTTGTTAATGGTTTTGCGGTCTTTAATAATAAGAT  
GAAAACAAAGTAAAAGCCTCTAAGAAATTTATCACTTTTATTGCTGA  
TGATAAAAAATGGGGACCAAAAGATGTTATACGTACAGGTGCTTTCC  
CAGTTAGAACATCATTGTTGGGATCTTTATAAAGGTGATAAACGTATG  
ATGAAGATTTCAAAATGGACTCAATATTATTCACCATATTACAACAC  
TATCGATGGATTTTCTGAAATGAGAACCTTATGGTTCCCAATGGTTCA  
ATCTGTATCCAATGGTGATGAAAAACCAGCAGATGCTTTGAAAGACT  
TACTCAAAAAGCAAATGATACCATTAAAAAAGCAGCTAAATAA

MSIKKSVIGFCLEAAALSMFACVDSSQSVMAAEKDKVEITWWAFPTFTQ  
EKAKDGVGTYEKKVIKAFEKKNPNIKVKLETIDFTSGPEKITTAIEAGTAP  
DVLFDAPGRIIQYGKNGKLADLNDLFTDQFIKDVNNKNIIQASKSGDKA  
YMYPISSAPFYMAFNKKMLKDAGVLKLVKEGWTTSDFEKVLKALKNK  
GYTPGSFFANGQGGDQGPRAFFANLYSAPITDKEVTKYTTDTKNSVKSM  
KKIVEWIKKGYLMNGSQYDGSADIQNFANGQTAFTILWAPAQPKTQAK  
LLESSKVDYLEVPFPSEDGKPDLEYLVNGFAVFNNKDENVKASKKFIT  
FIADDDKKWGPKDVIRTGAFPVRTSFGDLYKGDKRMMKISKWTQYYSPY  
YNTIDGFSEMRTLWFPMVQSVSNGDEKPADALKDFTQKANDTIKKA  
\*

ID-76 (Same as ID-39)

Clone 3-56

ATGAGGAAACGTTTTTCCTTGCTAAATTTTATTGTTGTTACTTTTATTT  
TCTTTTTCTTTATTCTTTTTCCGCTTTTAAAGGCCAAAGATTGTCAGGT

FIG. 1 CONT'D

SUBSTITUTE SHEET (RULE 26)

TGTTTATGCAAGTTTTCAAGGAGATCATTGGGACATTTGTAACGCATT  
TGATTTTCCGTATTTACATCGCTTTGATCTCATTAAAGGTAAAGAAAA  
TCAACTTTACTTTATAGGTTGTACAATTGCTAACAGTAAAGCCTACAC  
TGAGGATTGGAGTGATAAAGGCCGAATTTTTGTTGCTCGTTTTAATAC  
TCAAAACCATACATTGGAAGGATTGCAACAATTGCCTCAAACCTTTAT  
TAAAAAATCATGGATACTATGCCATTGAGGATGAAGGATATTCATTG  
ATTACTTCAGTAGAAGGGGTACTCAAACCTCACTTATCCAGAATTTTCT  
ACTACAGGCGACTGGCAATTAGAACGGCTTTTCGATGAGGAGACAAG  
CGATGTGGTGAAAGTGGATATTAATCAGGATGGTAAGGATGAGTATG  
TGATCATCCAAGGTTTTCATGGAGATCGTTTACGTATCTTCACTGAAG  
ATTCGGTCGAGAATTATTCATTATCCTGAAAAAACCCCATTTGGTC  
ACGCTATTTGGAGTGGTCGTTTACTTAATCAGACTTGTTTCGTATTCG  
GGTGGCGATCAGAAAAAGCAGAATTAAGGCTTTTCACTTTGTAGAT  
GGGCACTTGGTTTCAGAATTAGTAGATGCAAAAGCAGCTTCTAGTAA  
TGTCTTAGCTTTTGAAAAAGATGGAAAAGCTTATCTTTTCTCAGCCAA  
TAACGGACGTGGCGAAGTTGCTCTTTATCAATTAGTAAAATAA

MRKRFSLLNFIVVTFIFFFILFPLFKAKDCQVVYASFQGDHWDICNAFDF  
PYLHRFDLIKGENQLYFIGCTIANSKAYTEDWSDKGRIFVARFNTQNHT  
LEGLQQLPQTLKKNHGYIAIQDEGYSLITSVEGVKLKLTYPFSTTGDWQ  
LERLFDEETSDVVKVDINQDGKDEYVIIQGFHGDRLRIFTEDFGRELFHY  
PEKTPFGHAIWSGRLLNQTCFVFGWRSEKAELRLFHFVDGHLVSELVDA  
KAASSNVLAFEKDGKAYLFSANNGRGEVALYQLVK\*

FIG. 1 CONT'D

nucS1

Bgl II Eco RV

5'-cgagatctgatatctcacaaacagataacggcgtaaataag -3'

nucS2

Bgl II Sma I

5'-gaagatcttccccgggatcacaaacagataacggcgtaaataag -3'

nucS3

Bgl II Eco RV

5'-cgagatctgatatccatcacaaacagataacggcgtaaataag -3'

nucR

Bam HI

5'-cgggatccattatggacctgaatcagcggttgtc -3'

NucSeq

5'-ggatgctttgtttcagggtgtatc -3'

pTREP<sub>F</sub>5'-catgatatcggtacctcaagctcatatcattgtccggcaatgggtgtgggctttttttgttttagcggataa  
caatttcacac -3'pTREP<sub>R</sub>5'-gcggatcccccggttaattaatgtttaaacactagtcgaagatctcgcgaattctcctgtgtgaaatt  
gttatccgcta -3'pUC<sub>F</sub>

5'-cgccagggttttcccagtcacgac -3'

V<sub>R</sub>

5'-tcaggggggcgagcctatg -3'

V<sub>1</sub>

5'-tcgtatgttgtgtggaattgtg -3'

V<sub>2</sub>

5'-tccggctcgtatgttgtgtggaattg -3'

FIG. 2

pTREP-Nuc vectors allow cloning of genomic DNA into each frame with respect to the nuclease gene

(i)

pTREP1-nuc1 (EcoRV)	AAGTATCAGATCT-- <u>GATATC</u> --TCACAAACAGATAACGGCGTAAAT	Frame=+1
	.....▲.....	
pTREP1-nuc2 (Sma I)	AAGTATCAGATCTT <u>CCCCGGGA</u> -TCACAAACAGATAACGGCGTAAAT	Frame=+2
	.....▲.....	
pTREP1-nuc3 (EcoRV)	AAGTATCAGATCT-- <u>GATATC</u> CCATCACAAACAGATAACGGCGTAAAT	Frame=+3
	.....▲.....	
Nuclease Gene	TCACAAACAGATAACGGCGTAAAT	

Cloning site is indicated by an arrow

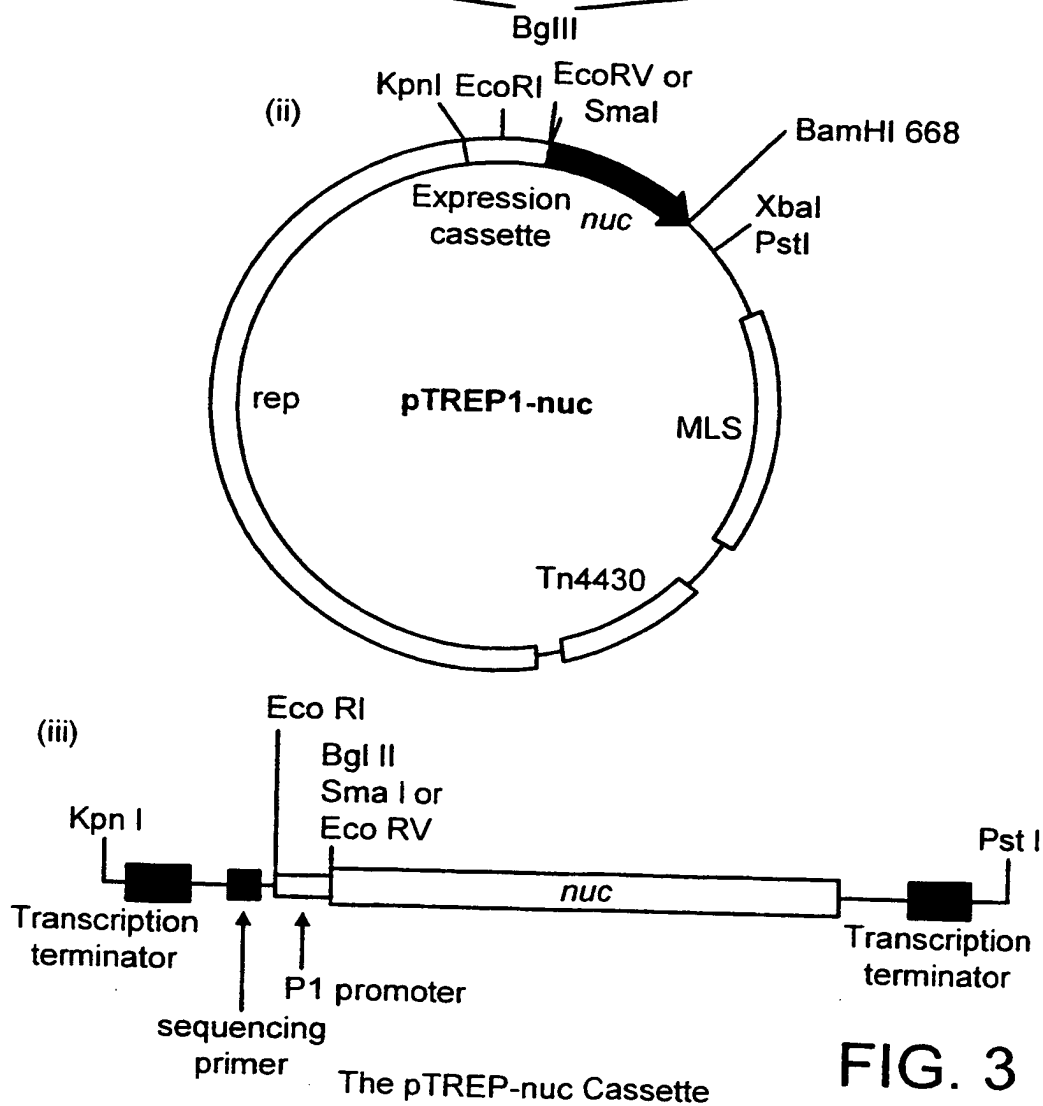


FIG. 3

## GBS Vaccination - Trial 1

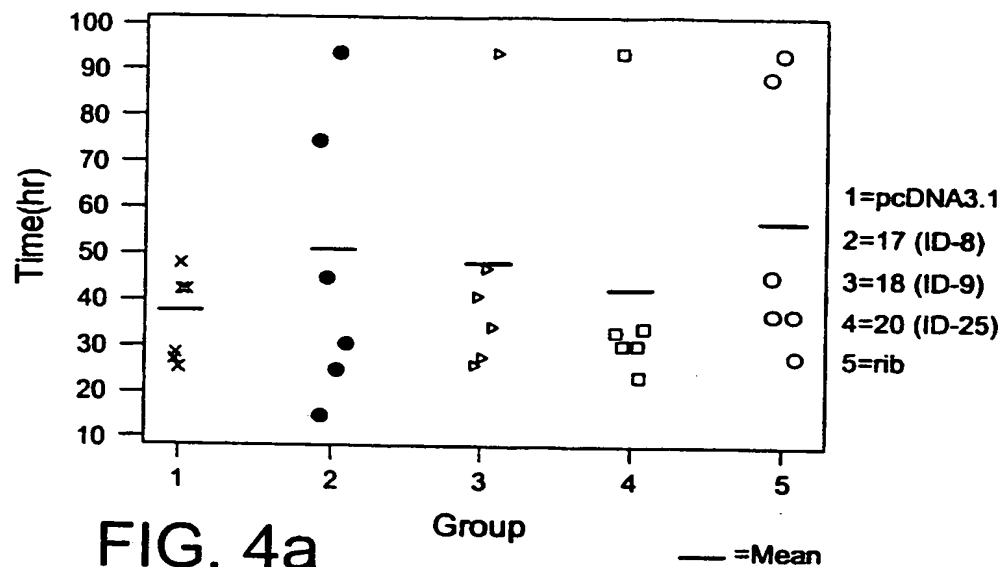


FIG. 4a

## GBS Vaccination - Trial 2

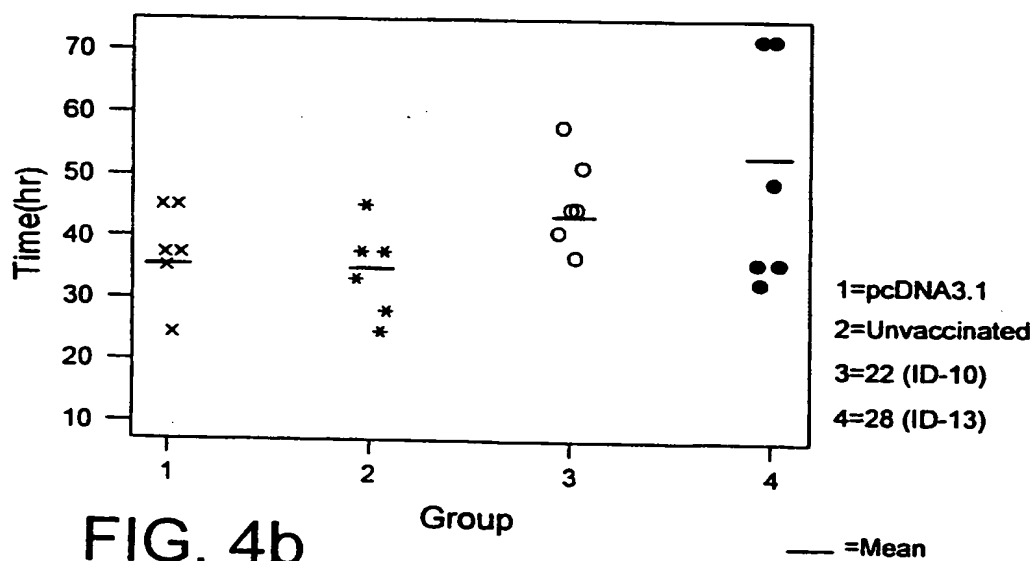


FIG. 4b

SUBSTITUTE SHEET (RULE 26)

## GBS Vaccination - Trial 3

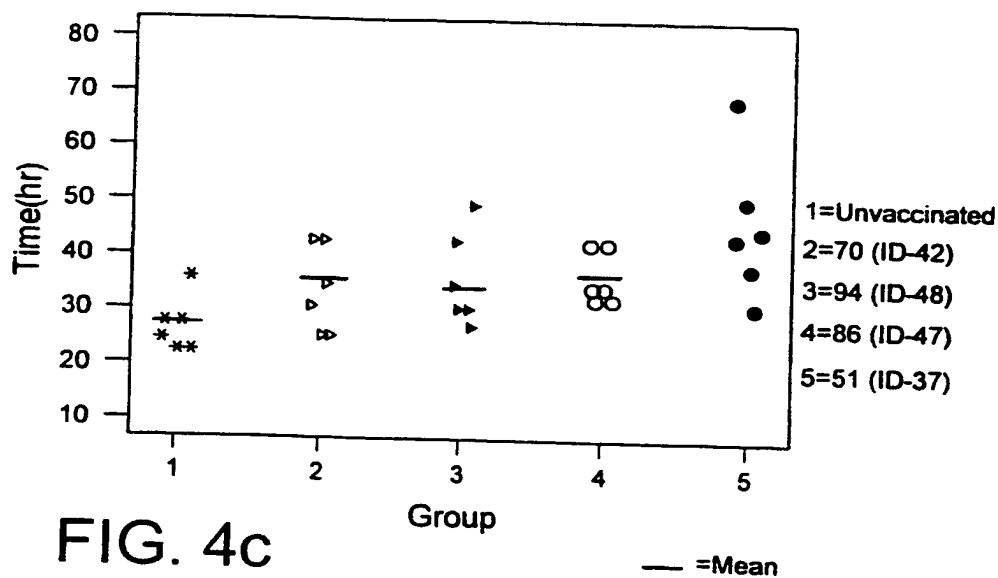


FIG. 4c

## GBS Vaccination - Trial 4

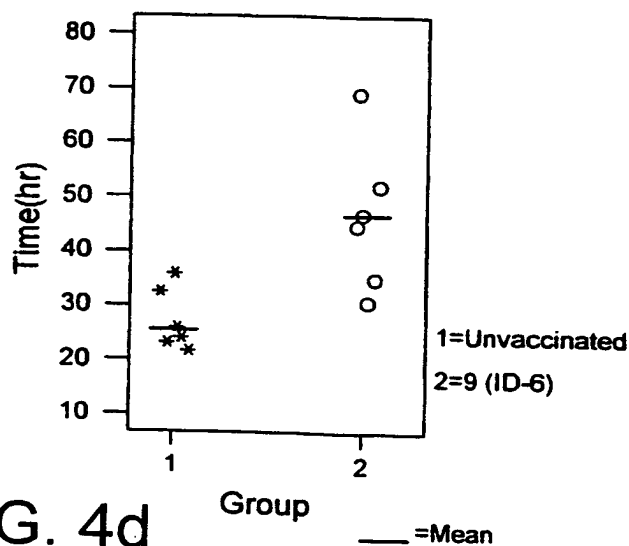


FIG. 4d

## GBS Vaccination - Trial 6

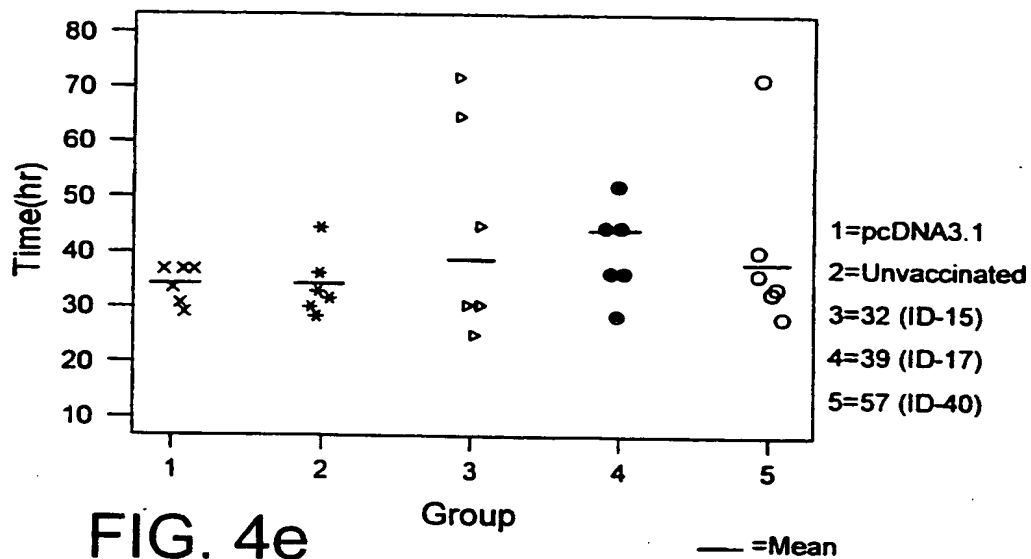


FIG. 4e

## GBS Vaccination - Trial 2

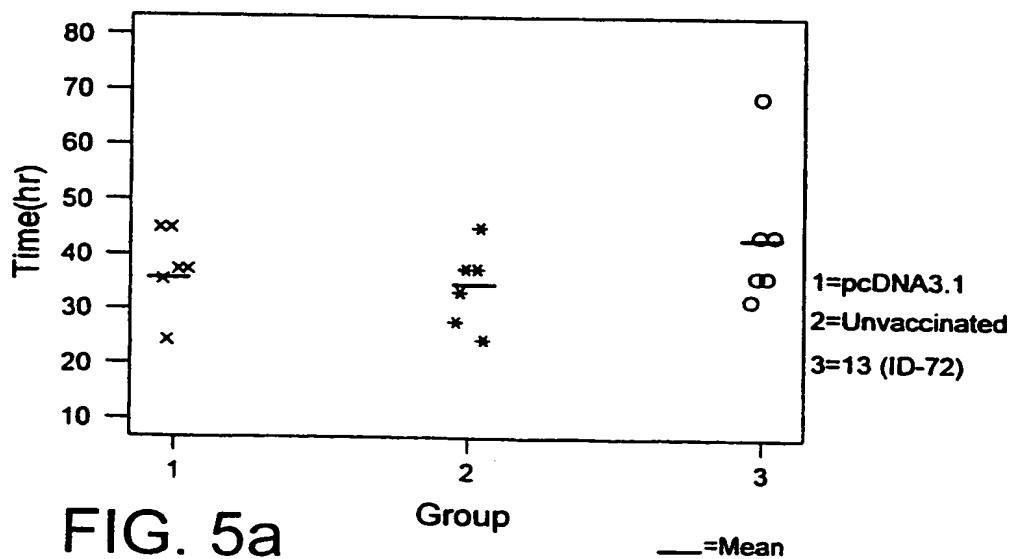


FIG. 5a

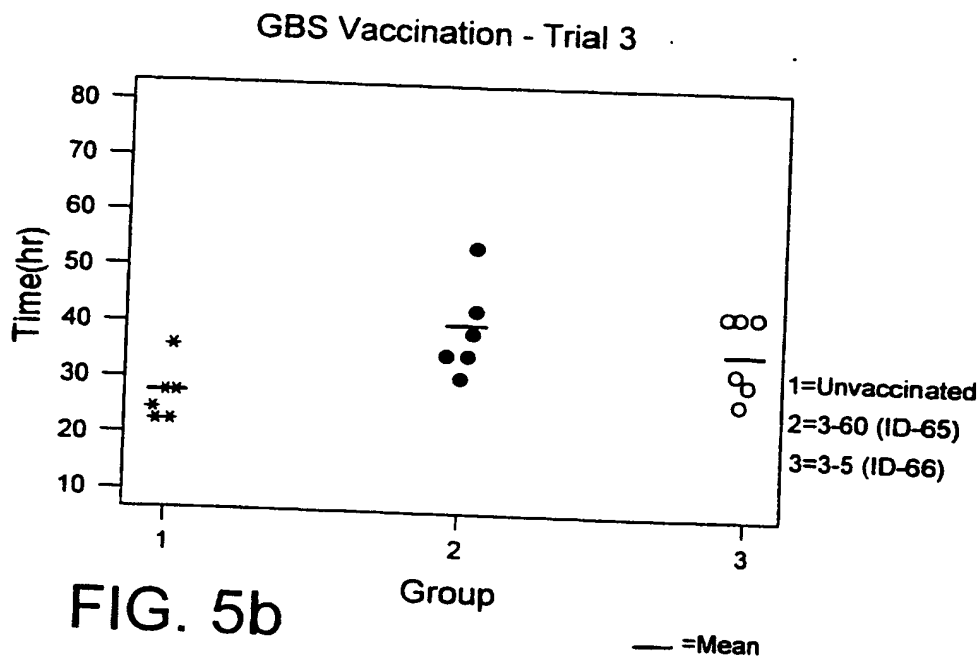


FIG. 5b

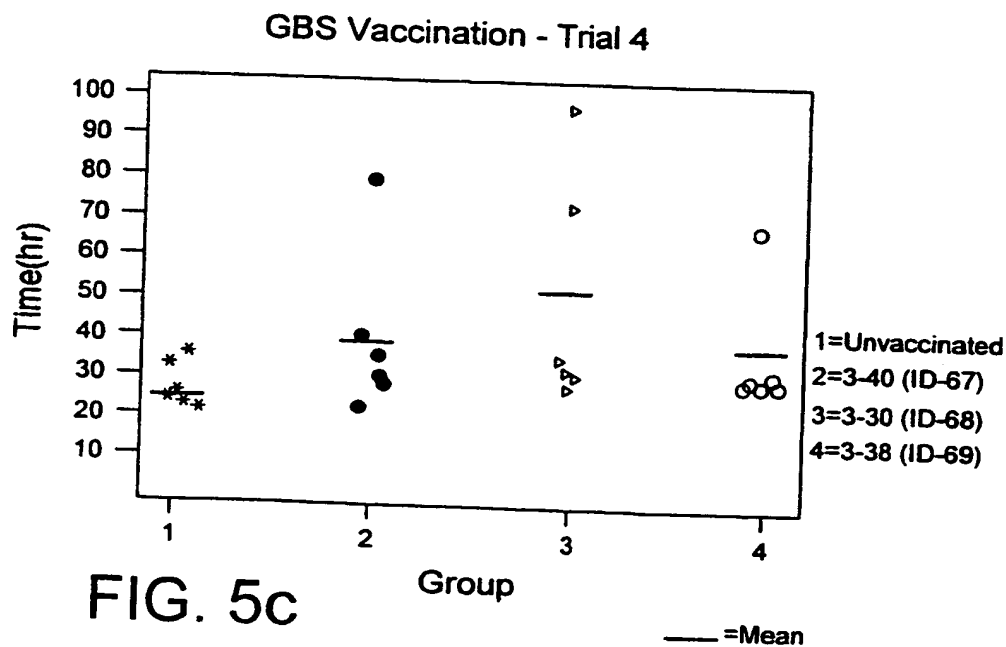


FIG. 5c

## GBS Vaccination - Trial 5

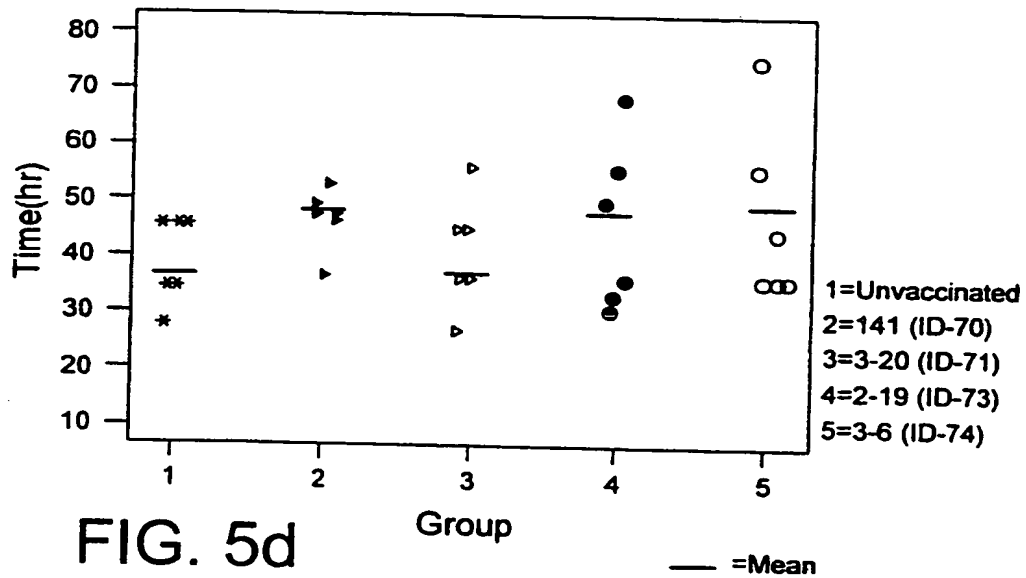


FIG. 5d

## GBS Vaccination - Trial 6

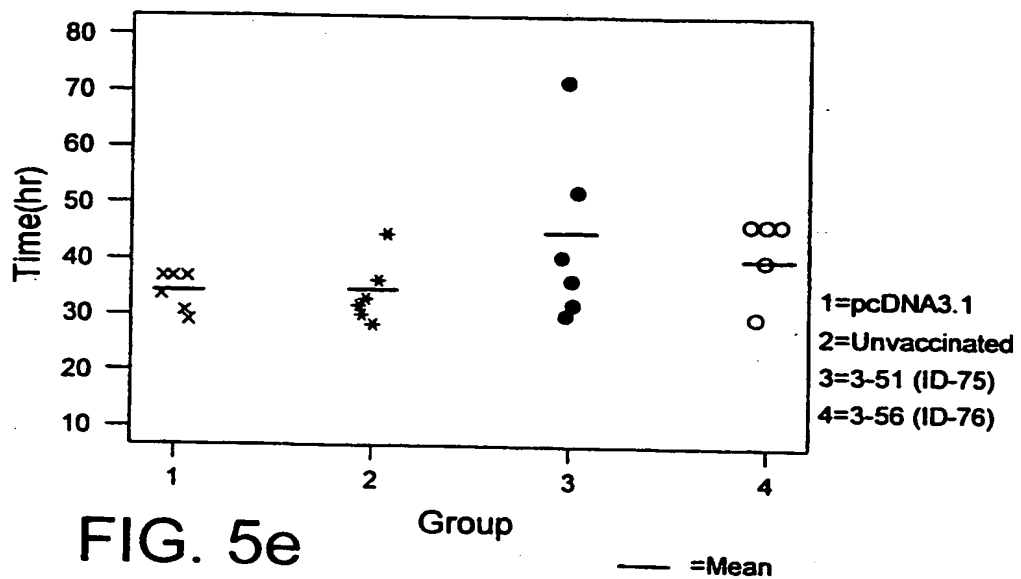


FIG. 5e

SUBSTITUTE SHEET (RULE 26)

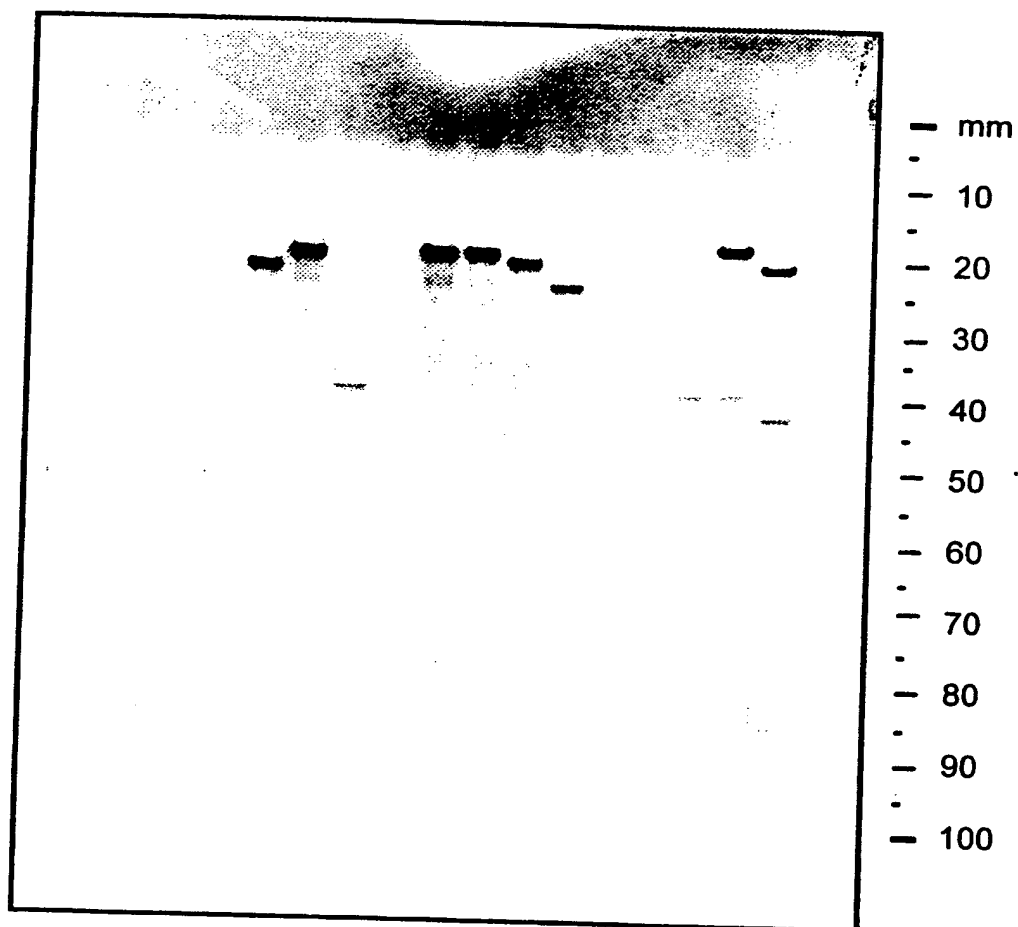


FIG. 6

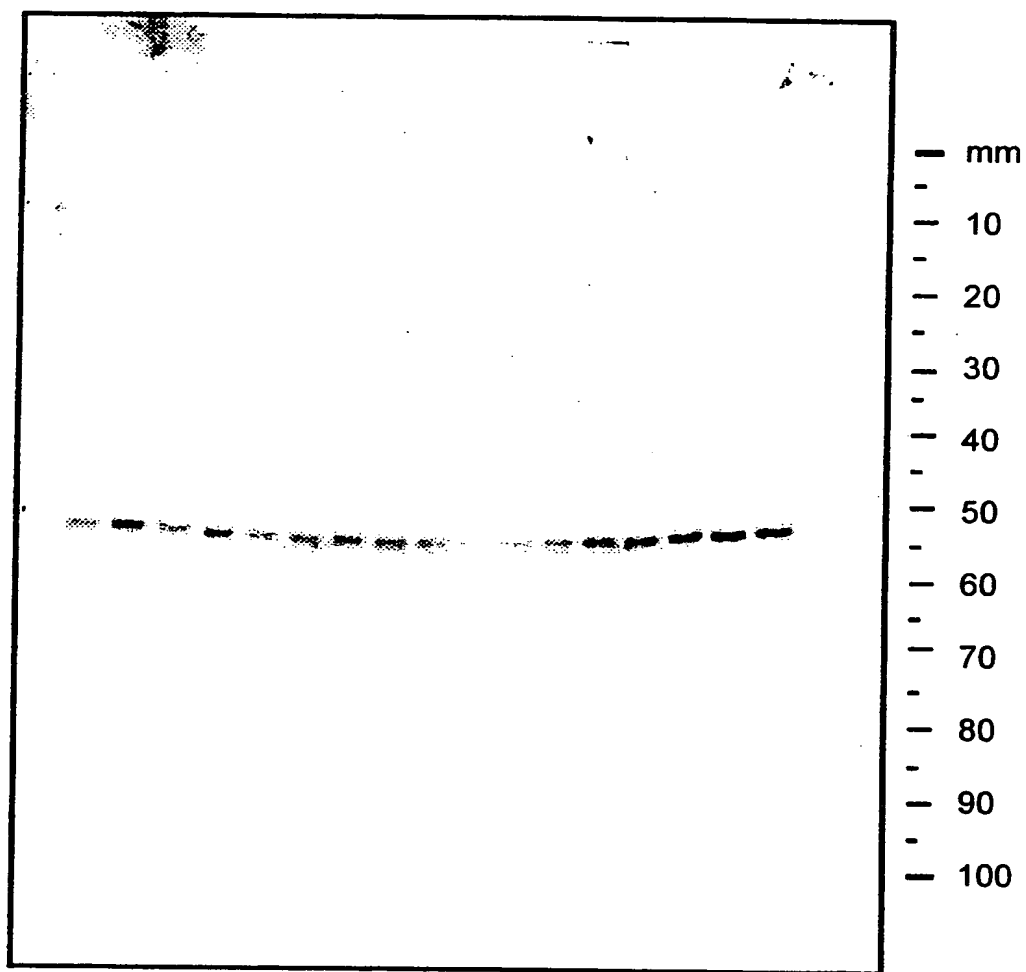


FIG. 7

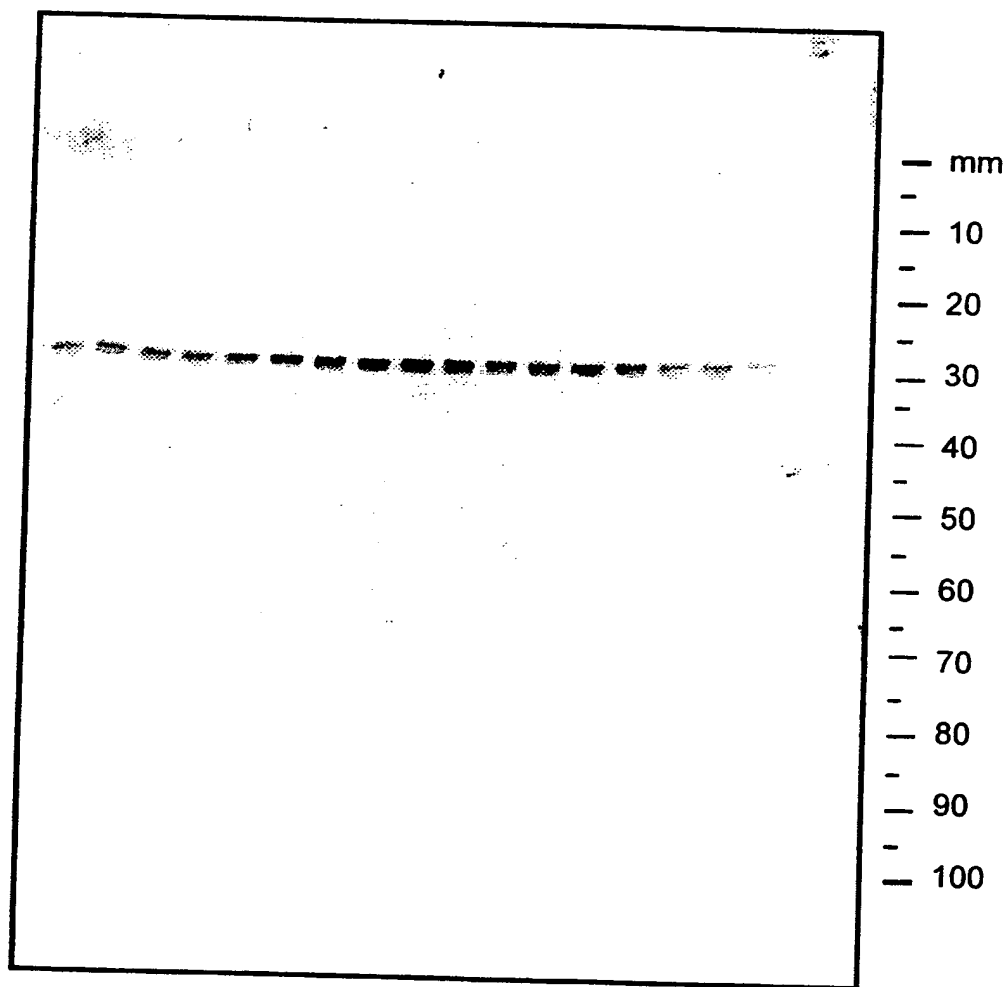


FIG. 8

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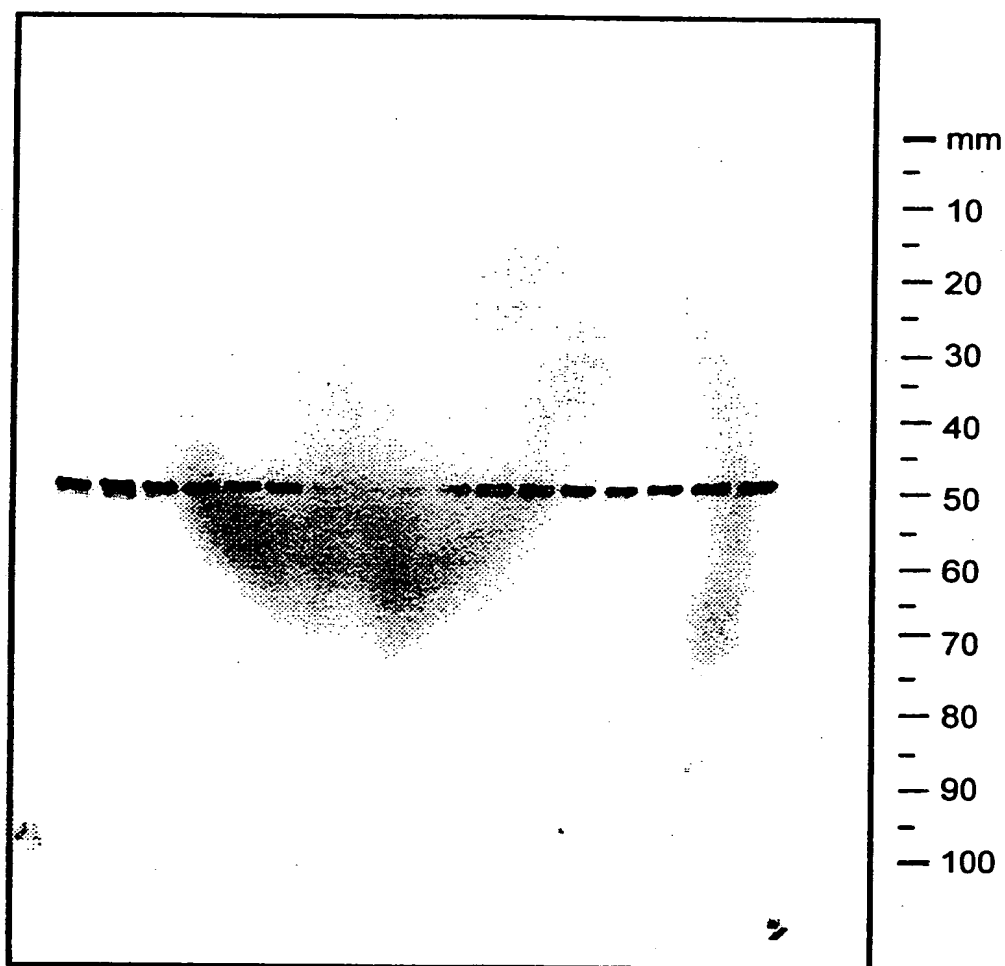


FIG. 9

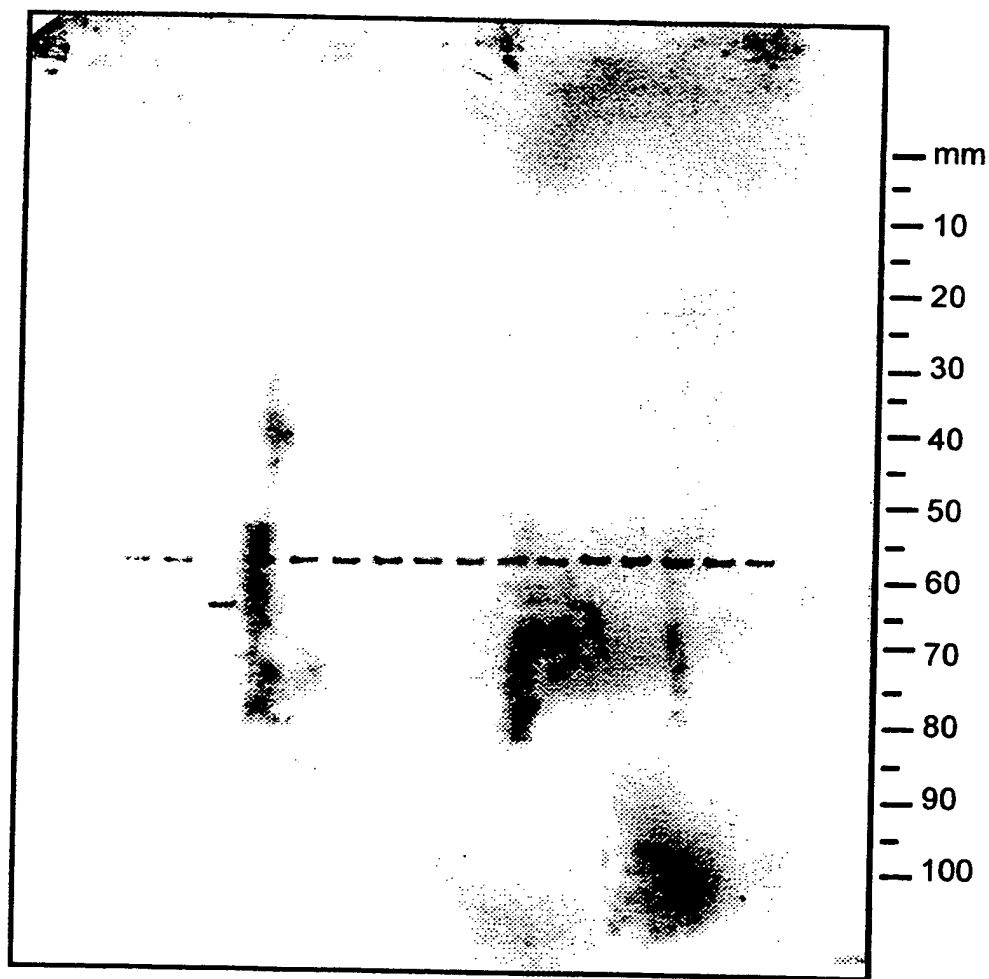


FIG. 10

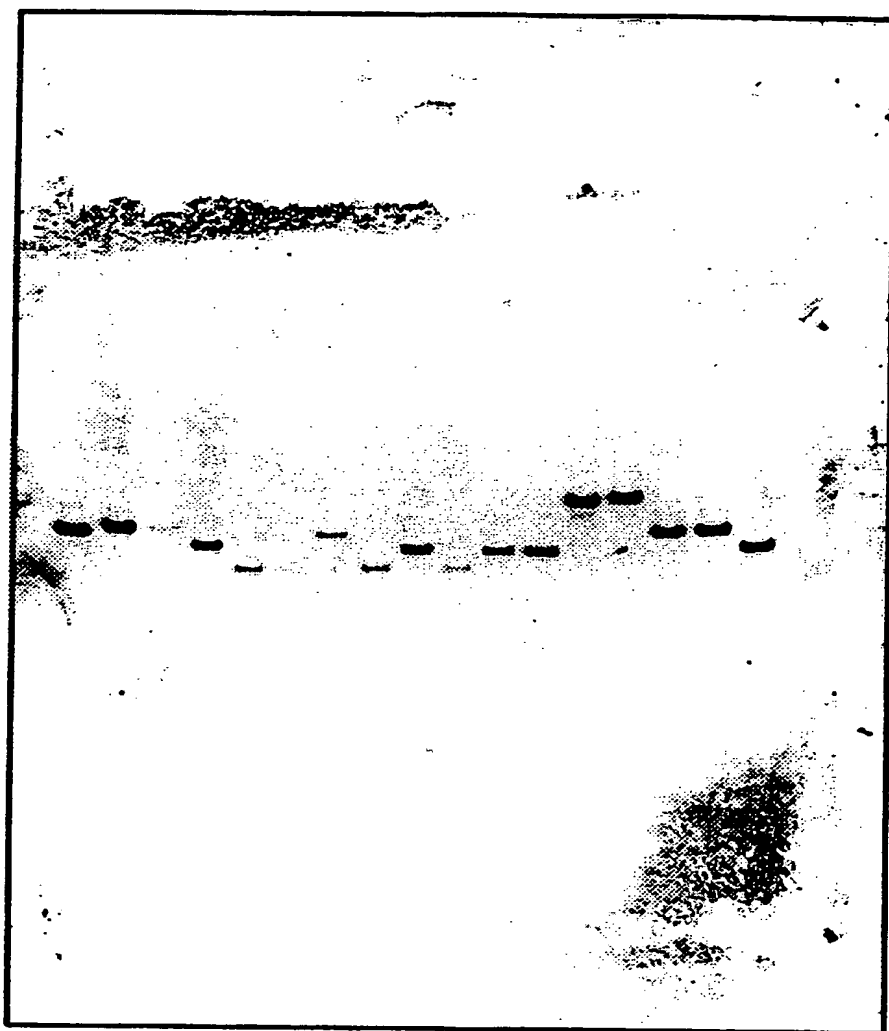


FIG. 11

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>C12N 15/31, 15/74, 15/62, 15/10, 9/16, 1/19, 1/21, C07K 14/315, 16/12, A61K 31/70, 39/09, G01N 33/53, 33/68, C12Q 1/68</b>	<b>A3</b>	<b>(11) International Publication Number:</b> <b>WO 00/06736</b> <b>(43) International Publication Date:</b> 10 February 2000 (10.02.00)
<b>(21) International Application Number:</b> PCT/GB99/02444 <b>(22) International Filing Date:</b> 27 July 1999 (27.07.99)  <b>(30) Priority Data:</b> 9816335.5 27 July 1998 (27.07.98) GB 60/125,163 19 March 1999 (19.03.99) US  <b>(71) Applicant (for all designated States except US):</b> MICROBIAL TECHNICS LIMITED [GB/GB]; 20 Trumpington Street, Cambridge CB2 1QA (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LE PAGE, Richard, William, Falla [GB/GB]; University of Cambridge, Dept. of Pathology, Tennis Court Road, Cambridge CB2 1QP (GB). WELLS, Jeremy, Mark [GB/GB]; Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA (GB). HANNIFFY, Sean, Bosco [IE/GB]; University of Cambridge, Dept. of Pathology, Tennis Court Road, Cambridge CB2 1QP (GB).  <b>(74) Agents:</b> CHAPMAN, Paul, William et al.; Kilburn & Strode, 20 Red Lion Street, London WC1R 4PJ (GB).		<b>(81) Designated States:</b> CA, CN, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>  <b>(88) Date of publication of the international search report:</b> 22 June 2000 (22.06.00)
<b>(54) Title:</b> NUCLEIC ACIDS AND PROTEINS FROM GROUP B STREPTOCOCCUS  <b>(57) Abstract</b>  Novel protein antigens from Group B <i>Streptococcus</i> are described, together with nucleic acid sequences encoding them. Their use in vaccines and screening methods is also described.		

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CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

# INTERNATIONAL SEARCH REPORT

National Application No

T/GB 99/02444

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C12N15/31	C12N15/74	C12N15/62	C12N15/10	C12N9/16
	C12N1/19	C12N1/21	C07K14/315	C07K16/12	A61K31/70
	A61K39/09	G01N33/53	G01N33/68	C12Q1/68	

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A61K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE TREMBL E.M.B.L. Databases Accession Number: Q54914, 1 November 1996 (1996-11-01) PODBIELSKI A ET AL: "ORF 1 AND ORF2 5' REGION" XP002133342 97.2% identity in 141 aa overlap with SeqIdNo.12 abstract</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	3,4

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

17 March 2000

Date of mailing of the international search report

11. 04. 00

Name and mailing address of the ISA

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Lonnoy, 0

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/02444

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 98 18930 A (HUMAN GENOME SCIENCES INC ;CHOI GIL H (US); HROMOCKYJ ALEX (US); J)  7 May 1998 (1998-05-07)  SP0020: 51.9% identity in 262 aa overlap with SeqIdNo.133  -&amp; DATABASE GENESEQ  E.M.B.L. Databases  Accession Number: W55078,  2 October 1998 (1998-10-02)  CHOI G ET AL: "Streptococcus pneumoniae SP0020 protein"  XP002133369  51.9% identity in 262 aa overlap with SeqIdNo.133  abstract</p>	3-18,23
P,X	<p>WO 99 16882 A (MEDIMMUNE INC)  8 April 1999 (1999-04-08)  -&amp; DATABASE GENESEQ  E.M.B.L. Databases  Accession Number: Y05766,  8 April 1999 (1999-04-08)  LUTTICKEN R ET AL : "Streptococcal adhesion mediator protein Lmb"  XP002133343  99.7% identity in 306 aa overlap with SeqIdNo.12  abstract</p>	1-18,23
A	<p>WO 94 10317 A (GEN HOSPITAL CORP ;BRIGHAM &amp; WOMENS HOSPITAL (US))  11 May 1994 (1994-05-11)  figure 6</p>	1-18,23
A	<p>MICHEL J L ET AL: "Cloned alpha and beta C-protein antigens of group B Streptococci elicit protective immunity"  INFECTION AND IMMUNITY,US,AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON,  vol. 59, no. 6, June 1991 (1991-06), page 2023-2028-2028 XP002107260  ISSN: 0019-9567  the whole document</p>	1-18,23
A	<p>LACHENAUER C S ET AL: "Cloning and expression in Escherichia coli of a protective surface protein from type V group B Streptococci"  ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY,US,SPRING ST., NY,  vol. 418, no. 418,  9 December 1997 (1997-12-09), page 615-618-618 XP002107261  ISSN: 0065-2598  the whole document</p>	1-18,23

## INTERNATIONAL SEARCH REPORT

national Application No

CT/GB 99/02444

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE SWISSPROT E.M.B.L. Databases Accession Number: P29850, 1 April 1993 (1993-04-01) PUYET A ET AL: " MALTOSE/MALTODEXTRIN-BINDING PROTEIN PRECURSOR" XP002125784 30.7% identity in 407aa overlap with SeqIDNo.2 abstract</p> <p>---</p>	1
A	<p>LARSSON C ET AL: "Experimental vaccination against group B streptococcus, an encapsulated bacterium, with highly purified preparations of cell surface proteins Rib and alpha" INFECT. IMMUN., vol. 64, no. 9, September 1996 (1996-09), pages 3518-3523, XP002125783 cited in the application</p> <p>---</p>	
A	<p>WO 95 06732 A (MASURE H ROBERT ;TUOMANEN ELAINE (US); PEARCE BARBARA J (US); UNIV) 9 March 1995 (1995-03-09)</p> <p>---</p>	
A	<p>DATABASE SWISSPROT E.M.B.L. Databases Accession Number: P42422, 1 November 1995 (1995-11-01) YOSHIDA K ET AL: "Hypothetical sensor-like Histidine Kinase in IDH 3' region" XP002133344 30.6% identity in 320 aa overlap with SeqIdNo.20 abstract</p> <p>---</p>	
A	<p>DATABASE SWISSPROT E.M.B.L. Databases Accession Number: P39845, 1 February 1995 (1995-02-01) TOGNONI A ET AL: "Peptide Synthetase 1" XP002133345 29.3% identity in 133 aa overlap with seqIdNo.26 abstract</p> <p>---</p> <p>-/--</p>	

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/02444

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE TREMBL E.M.B.L. databases Accession Number: P94374, 1 May 1997 (1997-05-01) YOSHIDA K ET AL: "HOMOLOGOUS TO MANY ATP-BINDING TRANSPORT PROTEINS" XP002133346 30.2% identity in 235 aa overlap with SeqIdNo.82 abstract</p>	
A	<p>--- WO 98 23631 A (KNOWLES DAVID JUSTIN CHARLES ; LONETTO MICHAEL ARTHUR (GB); SMITHKL) 4 June 1998 (1998-06-04) -&amp; DATABASE GENESEQ E.M.B.L. Databases Accession Number: W62662, 9 November 1998 (1998-11-09) BLACK M ET AL: "Streptococcus pneumoniae polypeptide" XP002133370 38.8% identity in 85 aa overlap with SeqIdNo.123 abstract</p>	
A	<p>--- WO 98 18931 A (DOUGHERTY BRIAN A ; HUMAN GENOME SCIENCES INC (US); ROSEN CRAIG A ()) 7 May 1998 (1998-05-07) -&amp; DATABASE GENESEQ E.M.B.L. Databases Accession Number: V52187, 7 May 1998 (1998-05-07) BARASH S ET AL: "Streptococcus pneumoniae genome fragment SEQ ID NO:54" XP002133371 61.8% identity in 2138 bp overlap with SeqIdNo.124 abstract</p>	
T	<p>--- WO 99 42588 A (BIOCHEM VACCINS INC ; BRODEUR BERNARD R (CA); CHARLEBOIS ISABELLE ()) 26 August 1999 (1999-08-26) -----</p>	

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/GB 99/02444

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:  
1-18 and 23 (all partially) as relating to inventions 1, 6, 10, 13, 35, 41, 62, 63 and 67
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Invention 1: claims 1-18 and 23 (all partially)

A Group B Streptococcus protein having a sequence as depicted in SeqIdNo.2, a fragment, derivative or variant of said protein; a nucleic acid molecule comprising or consisting of SeqIdNo.1, a nucleic acid molecule complementary to said sequence, a nucleic acid molecule encoding for the a derivative or fragment of said protein; a vector comprising said nucleic acid molecule and afferent recombinant DNA practices; an antibody to said protein; an immunogenic composition comprising said protein or said nucleic acid and applications thereof; a method or kit of detection of Group B Streptococcus comprising said protein, said antibody, or said nucleic acid molecule; a method of determining whether said protein represents a potential antimicrobial target which comprises inactivating said protein and determining whether Group B Streptococcus is still viable.

2. Inventions 2-69: claims 1-18 and 23 (all partially)

Idem as subject 1 but limited to each of the polynucleotide and polypeptide sequences as depicted in SeqIdNo:3-137, wherein invention 2 is limited to SeqIdNo:3 and SeqIdNo:4, invention 3 is limited to SeqIdNo:5 and SeqIdNo:6, ..., invention 58 is limited to SeqIdNo:115, ..., and invention 69 is limited to SeqIdNo:136 and 137.

3. Inventions 70: claims 19-22 (all totally)

A method for screening for DNA encoding bacterial cell envelope associated or surface antigens in gram positive bacteria comprising a reporter vector including the nucleotide sequence encoding the mature form of the staphylococcus nuclease gene and an upstream promoter region with DNA from a gram positive bacterium; said method wherein the reporter vector is one of the pTREPl-nuc vectors; said method wherein the gram positive bacterium is Group B Streptococcus, Streptococcus pneumoniae, Staphylococcus aureus or pathogenic group A streptococci; said vector which is one of the pTREPl-nuc vectors

For the sake of conciseness, the first and 70th subject-matters are explicitly defined, the other subject-matters are defined by analogy to the subject-matter of invention 1.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/02444

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9818930	A	07-05-1998	AU 5194598	A	22-05-1998
			AU 6909098	A	22-05-1998
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			WO 9818930	A	07-05-1998
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WO 9942588	A	26-08-1999	AU 2505999	A	06-09-1999
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